PRACTICAL ORGANIC
AND BIO-CHEMISTRY

R. H. A. PLIMMER

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PRACTICAL ORGANIC AND
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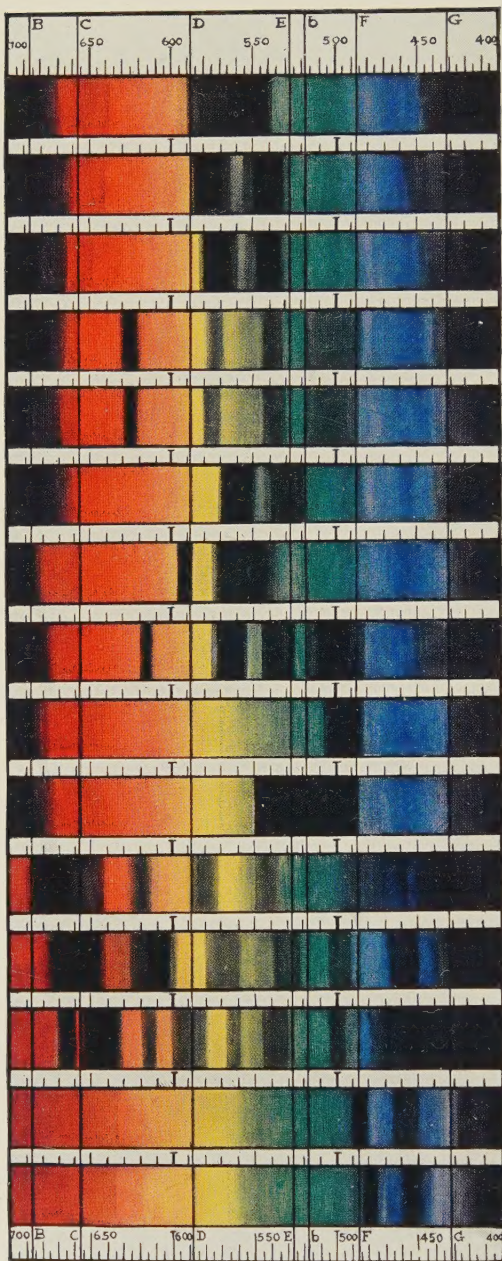
Hæmoglobin 1 : 100.

Oxyhæmoglobin 1 : 100.

Carboxyhæmoglobin 1 : 100

Methæmoglobin : neutral.

Hæmatin in acid alcohol.

Hæmochromogen in alkaline
solution, 1 : 100.

Hæmatoporphyrin in 1%
H_2SO_4.

Hæmatoporphyrin in 5%
NH_3.

Urobilin.

Uroerythrin.

Chlorophyll in living nettle
leaf.

a—Chlorophyll in ether.

b—Chlorophyll in ether.

Carotin in alcohol.

Xanthophyll in alcohol.

ABSORPTION SPECTRA.

PRACTICAL ORGANIC
AND BIO-CHEMISTRY

R. H. A. PLIMMER, D.Sc.

PROFESSOR OF CHEMISTRY IN THE UNIVERSITY OF LONDON, AT ST. THOMAS'S HOSPITAL MEDICAL SCHOOL

WITH COLOURED PLATE AND OTHER ILLUSTRATIONS IN THE TEXT

NEW EDITION
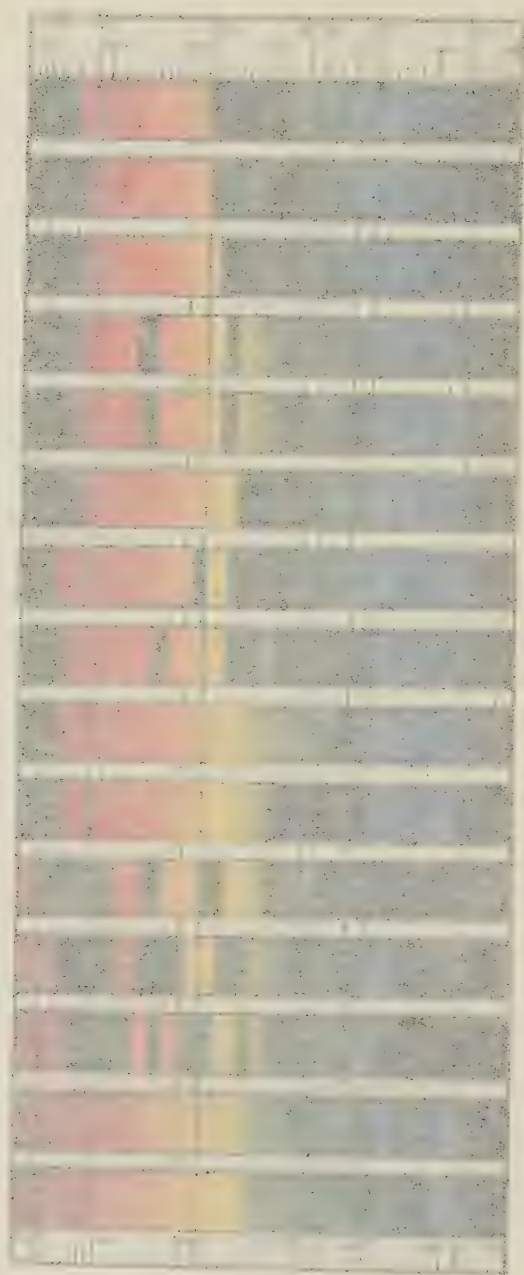
Hæmoglobin 1 : 100

Oxyhæmoglobin 1 : 100

Carboxyhæmoglobin 1 :

Methæmoglobin in acid

Hæmatin in acid aqueous

Hæmatogen in alkaline solution 1 : 100.

Hæmatoporphyrin in 1% HCl

Hæmatoporphyrin in 5% KHO

Urobilin

a - Chlorophyll in ether

b - Chlorophyll in ether

Carotin in alcohol

ABSORPTION SPECTRA.

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AND BIO-CHEMISTRY

BY

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PREFACE.

ORIGINALLY compiled as a handbook for practical work for medical students, this book has undergone various changes in the course of several editions. Further changes are again made. The chief alteration is in the enlargement of the theoretical part of organic chemistry, so that the book may serve both as a textbook and practical book on this subject. The advanced portions, not necessary in a general course, have been omitted, and additional matter has been added to the section on physiological chemistry. Every chapter has been revised so as to omit obsolete matter and to include recent work. The actual practical experiments are indicated, as before, by an asterisk.

The general treatment throughout all the editions, which is still retained, has been to consider organic chemistry as the basis of physiological chemistry, or rather the two as one sub-ject—Bio-chemistry.

R. H. A. P.

July, 1926.

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CONTENTS.

DEFINITIONS.

THE substances composing the organic material of all animal and vegetable cells, and the great majority of the products of vital activity, are mixtures of compounds of the element Carbon, in combination with only a few other elements, H, O, N, S, P.

From this mixture of substances the chemist has isolated numerous pure carbon compounds and prepared others. Many have still to be prepared in a pure condition. Carbon compounds have also been synthesised from their elements.

About 250,000 carbon compounds are now known. The possibility of their existence is due to the unique property which the element carbon possesses of being able to combine with itself; compounds are known which contain in their molecules from one up to sixty or more atoms of carbon directly joined together.

Of these 250,000 carbon compounds only a small number are directly concerned in vital processes.

The chemistry of all the carbon compounds is termed organic chemistry. It was originally believed that the natural carbon compounds could only be produced by "vital" force. Hence the term organic chemistry as distinct from mineral, or inorganic, chemistry. This belief was shattered by the synthesis of urea by Wöhler in 1828. It was followed by the synthesis of many other natural compounds.

The chemistry of those carbon compounds which are the constituents of living matter and how they are concerned in vital processes is physiological or biological chemistry.

The term physiological chemistry more frequently refers to the compounds and their functions in animals ; the term biological chemistry comprises the compounds and their functions in both plants and animals. The changes which they undergo and the functions which they fulfil in the living plant or animal form the subject of chemical physiology.

Though a distinction can be made between biological chemistry and chemical physiology, the two subjects are so closely interrelated that they are essentially only different aspects of the same subject. No biological change can be followed until a knowledge of the chemical properties of the substances involved has first been acquired. Chemical physiology is thus dependent on biological chemistry, or Bio-chemistry. Bio-chemistry is the branch of organic chemistry which deals with the natural organic compounds and with the functions of these compounds in nature.

RECOGNITION OF AN ORGANIC COMPOUND.

Organic compounds are distinguished from inorganic compounds by being combustible : on heating they generally char, sometimes take fire, and on prolonged heating completely burn away leaving no ash. Inorganic compounds when heated do not char and they leave a residue. A mixture of an organic compound and an inorganic compound will also char and leave a residue. There are a few exceptions to this general rule : oxalic acid and its salts amongst the organic compounds do not char on heating ; amongst the inorganic compounds the ammonium salts volatilise leaving no ash. An oxalate leaves a residue of the oxide of the metal with which it is combined.

The following experiments exemplify these statements :—

1. A small piece of paraffin wax heated upon platinum foil will melt, take fire, and will completely burn away leaving no residue.

2. A crystal of cane sugar heated in the same way will melt, char, and on further heating will disappear completely.

3. A few crystals of common salt heated on platinum foil will melt, and unless heated very strongly, e.g. with a blow-pipe flame, will remain as a solid white mass when allowed to cool.

4. A small piece of soap heated as above will char, the vapours evolved may take fire, and when the charred particles have all vanished a white or nearly white residue will remain.

Note.—It is in this way that substances composed of organic and inorganic matter are recognised. The composition of the inorganic residue is found out by the usual methods of inorganic analysis after the organic matter has been destroyed by heating.

5. No appreciable change will be seen on heating a little oxalic acid or an oxalate, e.g. calcium oxalate.

6. Ammonium chloride volatilises on heating and leaves no residue.

7. To prove the presence of carbon in oxalic acid or in an oxalate the substance is heated in a small glass tube and the gases evolved are passed into lime or baryta water. A precipitate of calcium or barium carbonate indicates the presence of carbon.

8. On heating ammonium chloride as in 7, there is no formation of carbonate.

9. A liquid containing matter in solution, such as urine, must first be evaporated to dryness.

CHAPTER I.

CRITERIA OF THEIR PURITY.

THE first step in the study of organic compounds is to separate them from one another and to prepare each of them in a state of purity. The pure substance can then be analysed and its chemical and physical properties ascertained.

In the study of the chemical properties of the compounds, other compounds are formed by their interaction. These compounds also require isolation and purification. The principal operations in organic and biological chemistry will thus consist in the isolation and preparation of pure compounds.

The methods of separating organic compounds are based upon differences in the properties of the substances under investigation. These differences are taken advantage of as much as possible ; sometimes they are so gross that the separation is simple, sometimes they are so small that the separation is of extreme difficulty, and in these cases a separation can only be effected when sufficient material is available.

Solid organic compounds are more numerous than liquid ; gases are comparatively rare.

I. PURIFICATION OF A LIQUID BY DISTILLATION— DETERMINATION OF THE BOILING-POINT.

A liquid is purified by distillation. The criterion of the purity of a liquid is its boiling-point. A pure liquid has a constant boiling-point.

A. Drying and Cleaning of Apparatus.

Since organic liquids are very frequently not miscible with water all the apparatus which is used with them must be dry.

The apparatus may be dried by placing it in an oven for some time or heating it carefully over a flame. On removal, to prevent deposition of aqueous vapour, a current of air is blown through it whilst

it is hot and during cooling. The current of air is most conveniently got from bellows, or by suction with a pump. The tubing, or if the glass vessel be narrow, a glass tube inserted in the tubing, is placed inside the vessel so that the farthest extremity is dried and cooled first.

Apparatus still wet with water may be rinsed with alcohol after draining as much water away as possible, then, after again draining, with ether. A current of air from the bellows or water-pump is drawn through to evaporate the ether. When no more ether is present and the vessel is not quite dry, it may be warmed in a luminous flame, and air driven through as above. It is important that all the ether be evaporated, since it may become ignited or form an explosive mixture inside the vessel.

Apparatus which contains charred matter may be cleaned by oxidising it away with potassium bichromate and sulphuric acid, or by heating in it a mixture of concentrated sulphuric and nitric acids, washing with water and proceeding as above to dry it.

B. Distillation of a Liquid.
Determination of its Boiling-point.

The liquid is placed in a clean, dry fractionating or distilling flask —a round-bottom flask with a side tube in its neck [1]—of suitable size so that only about half or at most two-thirds of the space is filled. Some small pieces of unglazed porcelain, or porous earthenware, or pieces of platinum, are added to ensure steady boiling without bumping. The neck of the flask is closed with a well-fitting cork [2] which is bored to carry a thermometer. The position of the thermometer is so adjusted that the bulb is just below or opposite the side tube and not touching the walls. The side tube is connected by a cork to a *clean, dry* condenser, which is supported by a clamp, and a slow stream of cold water is allowed to flow through the condenser. A receiver (flask) is placed at the other end of the condenser (Fig. 1).

A water condenser is not used for liquids boiling above 120°; the vapours are condensed by being passed through a simple tube (the inner tube of the above condenser). The vapours of liquids boiling at very high temperatures are condensed in the side tube of the distilling flask and no other condenser is necessary. If the vapours condense to

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[1] With liquids of high boiling-point the flask should have the side tube low down so as to prevent decomposition by the high temperature.

[2] Rubber corks are dissolved by many organic liquids and consequently are not used, except in special cases.

a solid on cooling, the solid is melted by a flame so that the liquid runs into the receiver and does not block up the side tube.

Liquids boiling below 100° are heated on a water-bath, liquids boiling above 100° are heated directly with a flame, which is moved round and round under the bottom of the flask until boiling begins. When boiling commences it must be kept on continuously and vigorously and not interrupted by the removal of the flame or by draughts.

When the vapour from the boiling liquid reaches the thermometer, the temperature is seen to rise rapidly, and then becomes stationary at a definite temperature. This is the boiling-point of the liquid. Drops of condensed liquid are usually seen to fall from the end of the
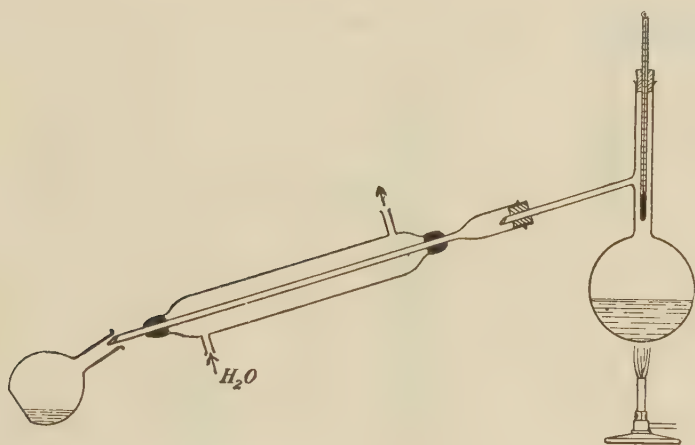
FIG. 1.

thermometer into the flask. The heating is continued until all the liquid boiling at this temperature has distilled over into the receiver. The portion remaining in the distilling flask contains the impurities.

Towards the end of the distillation the thermometer may be seen to rise slowly and the last portion to distil 1° higher than the first portion. Most pure liquids boil over a range of ·5° or 1° or sometimes more.

Chloroform and aniline may be used as examples.

When insufficient liquid is available for distillation its boiling-point can be determined by placing it in a test tube and heating it through an opening in a sheet of asbestos. The thermometer is held in the vapour.

C. Distillation *in vacuo.*

Liquids which boil at high temperatures and decompose on distillation under atmospheric pressure can frequently be distilled under reduced pressure.

The liquid is distilled from a fractionating flask, the side tube of which is inserted in another fractionating flask, or other stout vessel with a side tube, which acts as receiver. This is kept cold by allowing a stream of cold water to run over it ; the water is collected in a funnel, which serves as a support to the receiver and it runs thence to the waste. The vacuum is produced by connecting the side tube of the receiver with a water pump or a mechanical pump. A gauge is in connection between the apparatus and the pump by a T piece so that the pressure can be ascertained.

To ensure continuous ebullition without bumping a slow stream of air, or carbon dioxide from a Kipp apparatus if the liquid tends to oxidise or decompose in the air, is passed through the liquid by inserting in the cork a tube
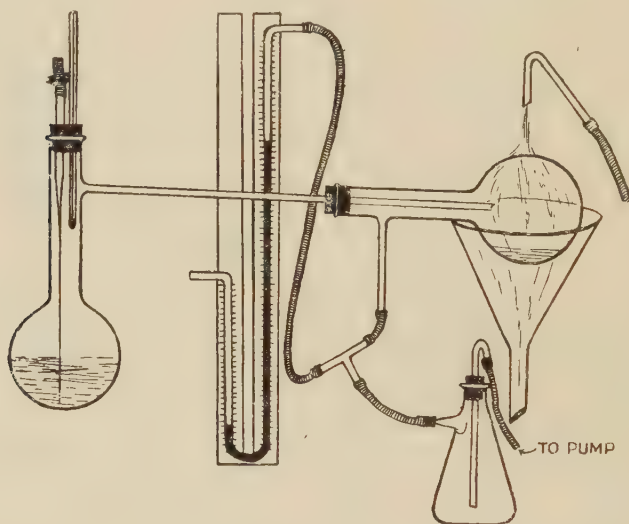
FIG. 2.

with a long capillary which reaches almost to the bottom. The supply of air is regulated by means of a screw pinch on a piece of pressure-tubing placed on the end of the glass tube. The apparatus is shown in Fig. 2.

A distilling flask with a double neck as in Fig. 5 (p. 10) is more convenient than a simple distilling flask ; the neck with the side tube carries the thermometer, the other neck the capillary.

The heating of the flask during distillation *in vacuo* is best effected by means of a bath ; overheating is prevented, and the flask and its contents can be completely immersed which ensures a uniform temperature.

Rubber corks are used in vacuum distillation as they more easily prevent leakage in of air. If they are attacked by the vapours of the liquid they may be protected by placing a piece of cork between their ends and the vapours.

II. SEPARATION OF LIQUIDS.

A. **Mechanically.**

*   (*a*) Two liquids, if they are not miscible, are easily separated from one another. They are placed in a tap funnel or separating funnel (Fig. 3), either cylindrical or pear-shaped according to the volume, the stopper is removed and the heavier liquid run out and collected. The lighter liquid is poured out through the top so as to avoid contamination by coming in contact with drops of the other liquid remaining in the stem.

(*b*) Two liquids, if they are miscible, may not both be soluble in the same solvent; such a solvent must not be miscible with one of the constituents. On shaking the mixture with the solvent in a separating funnel, the mixture will separate into two layers.

In shaking up two liquids in a separating funnel, the stopper of the funnel is held in the palm of one hand, and as usually an increase of pressure occurs, especially if ether and water be the liquids, the tap of the funnel is occasionally opened after allowing the liquids to settle at the other end. After thoroughly shaking, the liquids are allowed to separate, the stopper is removed, and the heavier

FIG 3.

liquid is run out. The insoluble liquid is shaken up once or twice more with more solvent, and separated.
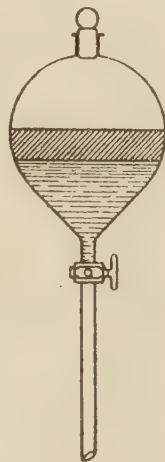
Drying of Organic Liquids.

If water has been in contact with the organic liquid, a small quantity will be dissolved by that liquid. The organic liquid is wet. It is dried by shaking it, or allowing it to stand for 12-24 hours with solid calcium chloride, or sodium sulphate, or potassium carbonate. It is then filtered and distilled.

*   As an example, a mixture of equal parts of alcohol (50 c.c.) and chloroform may be made. On shaking up with two volumes of water in a separating funnel, the chloroform separates and sinks. It is removed and the other constituents are poured out. The chloroform is returned and shaken once more with water. It is run out and the moisture is removed by shaking or standing with some calcium chloride from which it is filtered and then distilled.

B. Fractional Distillation.

A mixture of two or more miscible liquids is usually separated by fractional distillation if their boiling-points differ by 20-30°.

The mixture is distilled as described previously (p. 4), preferably from a flask with its side tube high in the neck and the thermometer is carefully watched. The first portion which distils will consist mainly or entirely of the more volatile constituent which has a higher vapour pressure; the last portion will consist of the less volatile constituent having a lower vapour pressure or higher boiling-point. Between these portions there may be a small intermediate fraction consisting of a mixture of the liquids. The three portions, or fractions, are collected in separate receivers.

E.g.—

A mixture of equal volumes of chloroform and aniline will give on fractional distillation a fraction boiling at 61° (almost pure chloroform), an intermediate fraction, and a final fraction boiling at 183° (almost pure aniline). During the distillation of such a mixture when the boiling-point of a constituent exceeds 120° the water should be run out of the condenser.

Redistillation of the first and last fractions will give each of them in a state of purity.

If a mixture of liquids contains constituents which have boiling-points fairly close to one another, the fractionation must be repeated several times until each fraction is found to have a constant boiling-point.

The separation of such a mixture is greatly facilitated by the use of a fractionating column or still-head. This is simply a device to lengthen the neck of the distilling flask so that the higher boiling fractions are exposed to the air and condensed before they reach the condenser and run back into the flask. Numerous forms have been invented; two efficient forms are those of Hempel and of Young (pear still-head, Fig. 4).

The former consists of a glass tube filled with glass beads and a side tube. The latter consists of a piece of glass tubing upon which are blown 2, 3, 4 or more bulbs of a pear shape, and a side tube. The liquid is placed in a round-bottom flask, the fractional column inserted, and this in turn connected by its side tube to the condenser. The
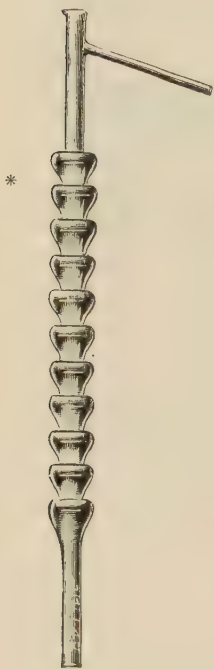
FIG. 4.

mixture in the flask, to which several small pieces of porous earthen-
ware have been added, is heated over a gauze at such a rate that the
condensed liquid comes over drop by drop. Fractions are collected at
5° or 10° ranges of temperature.

The redistillation of each fraction is carried out as follows : In
the apparatus which has been washed out and dried, fraction I, which
boiled, say, at 90-95°, is put and distilled till the thermometer shows
95°, when the distillation is stopped and fraction II, 95-100°, is added
to the remainder in the flask. On distilling, fractions below 95° are
collected in the first receiver and the second fraction is distilled till
the temperature reaches 100°. Distillation is stopped and the next
fraction added. The process is continued until all the fractions have
been redistilled.

A mixture of benzene, toluene, and xylene, such as occurs in coal
tar, may be taken in illustration.

Constant Boiling Mixtures.

It frequently happens that two liquids form a mixture which has
a constant boiling-point and behaves like a single liquid. Such a
mixture cannot be separated by fractional distillation, although more
or less separation may be possible by distilling at a different pressure.

Such a mixture may have a boiling-point which is lower than
either of its constituents, or higher.

Excess of either constituent in the mixture beyond that forming
the constant boiling mixture can be separated by fractional distilla-
tion and it will distil over either before or after the mixture according
to the boiling-points. Such constant boiling mixtures are mixtures
of ethyl alcohol and water, methyl alcohol and acetone, benzene and
alcohol, pyridine and water, water and formic acid, chloroform and
acetone. They can only be separated by chemical means.

Fractional Distillation *in vacuo.*

The same apparatus as described above for distillation *in vacuo* can be
used for fractional distillation *in vacuo*, but as each fraction distils the
apparatus must be disconnected so as to insert a new receiver. To avoid
releasing the vacuum and disconnecting the apparatus, and to allow fractional
distillation to proceed continuously several contrivances have been suggested.

An apparatus of the type in Fig. 5 is the most convenient. By means of
the several taps the receiver can be shut off and its vacuum released, whilst
distillation continues and the fraction collects in the bulb. The fresh
receiver is exhausted whilst the taps to the bulb and distilling flasks are
closed ; no great decrease of vacuum occurs as the small receivers are
rapidly exhausted.

If necessary, the bulb and receiver can be cooled by a stream of cold water, or by immersing the receiver in ice or a freezing mixture.

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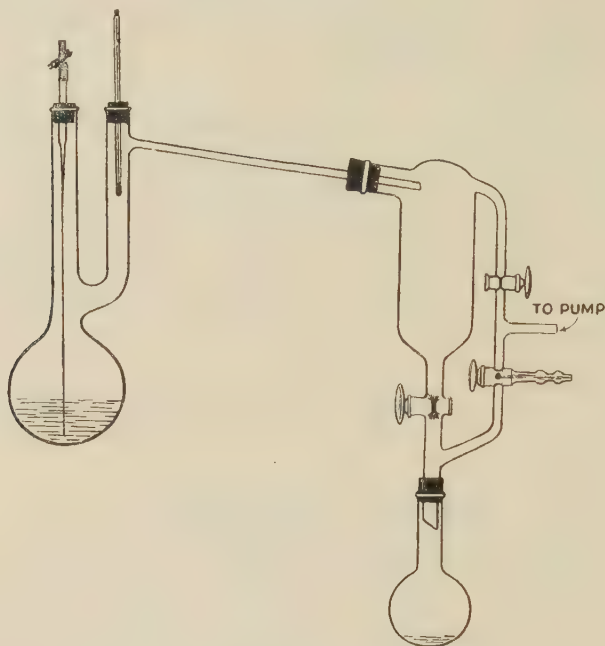
Fig. 5.

C. **Steam Distillation.**

Very frequently a separation of a liquid or a solid from a mixture can be effected by the process of steam distillation. The liquid, or solid, has usually a higher boiling-point than water, but the vapours of the liquid and of the water do not interfere with each other. The effect of the steam is to reinforce the vapour pressure of the liquid, so that the liquid distils with water under atmospheric pressure at a lower temperature.

Steam is generated in a large flask, or tin can, which is provided with a cork carrying a long safety tube about 80 cm. long, reaching almost to the bottom, and with a delivery tube. The flask is half-filled with water. The delivery tube, when the steam is ready, is connected to the flask containing the mixture. This flask is placed in a sloping position so as to prevent splashing of its contents and mechanical carrying over of substance into the condenser. The steam is passed into the bottom of the vessel by a tube which is bent so that its end lies in a vertical position, and to prevent condensation of the steam the flask is also heated. The steam and other vapour reach a long

condenser by which water and substance are condensed and are col-
lected in a receiver (Fig. 6).

The separation of substance and water is effected by filtration if
solid, by simple separation if liquid, or by extraction with a solvent
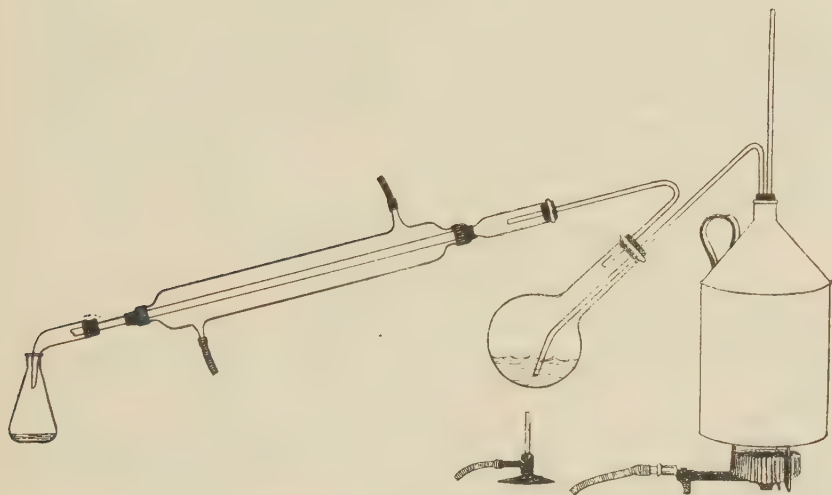
Fig. 6.

such as ether, chloroform, etc. The solvent is then dried, removed by
distillation, and the substance or substances obtained by distillation
or fractional distillation.

D. Evaporation of Liquids.

Only aqueous solutions can be evaporated over a flame, and the
evaporation should be completed over a water-bath to prevent charring
the substance as it becomes concentrated. Evaporation over a flame
must be carefully watched to prevent charring and also to avoid spurt-
ing when the solution begins to concentrate.

Most organic liquids which are used as solvents are readily inflam-
mable and must not be brought near a flame.

When a considerable amount of solvent is present it is removed by
distillation and in cases where the solvent (ether, acetone, ligroin, etc.)
is very readily inflammable the distillation must be carried out on
a water-bath, heated by a flame specially protected by a gauze, or by
steam, or electrically. If the bath be heated by a burner, the end of
the condenser should be placed as far away as possible and a sheet
of cardboard or asbestos interposed between the burner and receiver.

Not only is evaporation by distillation absolutely necessary with

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inflammable liquids, but also it is economical.   The solvent is recovered and after purification can be used again.

When only small quantities of liquids up to 25 c.c. require evaporation they are set aside, away from flames, and allowed to evaporate spontaneously, or · they may be put upon a warm water-bath, with the flame extinguished.

Another common procedure is to evaporate small quantities by placing them in a vacuum desiccator and exhausting.   The evaporation is greatly accelerated if the liquid be previously warmed on a water-bath and whilst warm put into the desiccator.

III. PURIFICATION OF A SOLID BY CRYSTALLISATION.

The majority of solid organic compounds are crystalline, but many of the complex solids, such as starch, glycogen, various proteins, which belong to the class of substances termed colloids, have not yet been prepared in a crystalline form and are only known in an amorphous state.   Others, such as the fats, though obtainable in a crystalline form, are mixtures of closely related substances, and it is extremely difficult to separate them into individual compounds.

The separation of a solid in a crystalline form is essential to its preparation in a pure condition.   Its recrystallisation, when once obtained in a crystalline form, will lead to its preparation in a state of purity.

The criterion of the purity of a crystalline solid compound is its melting-point.   A pure solid organic compound melts sharply at a definite temperature.   An impure organic compound does not ·melt sharply and it melts at too low a temperature.   The melting-point is found to rise on recrystallisation.

(a) Choice of Solvent for Crystallisation.

The purification of a solid by crystallisation depends very largely upon the choice of a suitable solvent.   The best condition for purification is very slight solubility in the cold solvent and ready solubility in the boiling solvent.   A hot saturated solution of the solid will deposit the greater part of the solid in a crystalline condition on cooling, and if the solution be allowed to cool slowly the crystals which are deposited will be more regular than if the solution be cooled rapidly.

In order to ascertain the solubility of a substance in a solvent the substance must be in a fine state of division.   A small quantity of the substance is finely powdered in a watch glass with a glass rod, or better in a small agate

mortar with a pestle. A few milligrams of the substance are then placed in a small test tube, a few drops of solvent are added, and the solid well stirred or shaken with it. If the solid is apparently not soluble in this amount of solvent more is gradually added, and so it can be determined whether the substance is easily soluble, moderately soluble, or insoluble in the cold liquid.

The solvent in those cases where the substance is slightly soluble or insoluble is now heated; if the solid dissolves easily more is added until the solution is saturated; if not, more solvent is added so as to bring, if possible, the solid into solution. The solution is cooled by holding under running water and it is noticed how much of the solid crystallises out. If a considerable quantity separates out, the solvent will probably be suitable for recrystallising larger quantities.

Sometimes crystallisation does not occur spontaneously on cooling, but it may be started by scratching the sides of the test tube or by adding a crystal of the solid.

The following solvents are most frequently used :—

(1) water	(4) benzene	(7) glacial acetic acid
(2) alcohol	(5) chloroform	(8) methyl alcohol
(3) acetone	(6) ligroin	

* For example, benzoic acid may be crystallised from water, and urea from alcohol, using a reflux condenser.

(b) Recrystallisation.

If the suitable solvent has been found to be water, or glacial acetic acid, or a liquid which is not inflammable and boils at a fairly high temperature, the recrystallisation may be carried out in a flask or beaker heated over a gauze.

If the suitable solvent has been found to be alcohol, acetone, ligroin, benzene—liquids which are volatile and inflammable—the recrystallisation must be carried out in a flask to which is attached a reflux or inverted condenser, as in Fig. 7.

Solvents boiling below 90° are heated on the water-bath, above 100° over a flame through a wire gauze and with an air condenser (inner tube of condenser or a tube about 80 cm. long by ·5-1 cm. in diameter) as reflux.

$H_2 0$

FIG. 7.

(i) Solution.

The substance is powdered finely and placed in a flask or beaker with a small quantity of solvent. Excess of solvent must be avoided
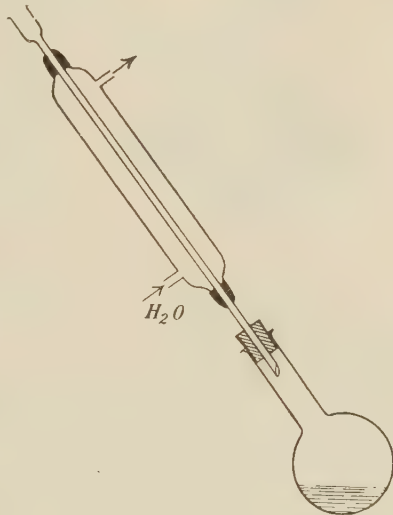
as the object is to prepare a hot saturated solution.   The solvent is boiled.   If, after boiling for some time, a considerable amount of solid remains, more solvent is cautiously added (through the condenser) and the boiling continued ; solvent is added until the whole of the solid, except insoluble matter, is dissolved.   It should be noted that the last portions of a solid often only dissolve with difficulty and fresh solvent should not be added too soon.

(ii) **Filtration.**

Particles of insoluble impurity are now filtered off by *rapidly* filtering the hot solution preferably through a pleated or fluted filter paper.

A filter paper is first folded into quarters in the ordinary way. Each quarter is then bisected by folding towards the hollow of the central fold, and each of these divisions is bisected again in such a way that the hollows and ridges alternate (Fig. 8). More pleats are obtained in the same way by bisecting the divisions and folding alternately.   The paper is thoroughly pressed to make the pleats permanent.
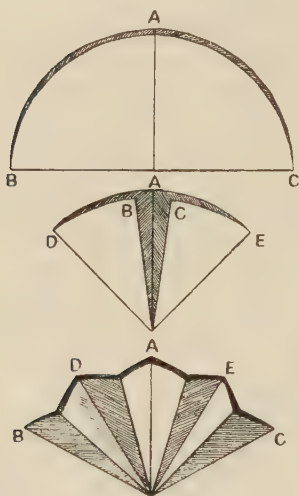
Very frequently the solution is very concentrated and begins to crystallise immediately filtration is commenced.   To avoid this a funnel with a very short stem, or without stem, is used, and it is previously heated in an oven or by passing through a flame ; the solution is filtered whilst it is still hot.

If crystallisation of solid should commence during the filtration, the funnel and paper are placed over the flask, the paper pierced and the particles washed with a little solvent into the flask and the solution again boiled up.

Fig. 8.

The filtrate is collected in a beaker of such a size that it is not filled more than two-thirds.

When the tendency to crystallise immediately is very pronounced the filtration must be carried out through a funnel heated by steam.   The funnel may be surrounded by coils of metallic piping through which steam from a generator is passed, or it may be enclosed in a larger metal funnel with two walls between which there is water and from the outer of which there is a projection for heating the contents by a flame.   Care must be taken that inflammable liquids do not become ignited if this form of hot-water funnel be used.   The water can be raised to boiling and the flame removed.

If crystals begin to separate before the filtration is completed, it is best to heat the filtrate until solution is again effected. To exclude dust and prevent evaporation, the beaker is covered with a clock glass with its convex side uppermost. Condensed drops of solvent will then run towards the side and not drop into the liquid and disturb the formation of the crystals. The solution is set aside in a cool place to crystallise.

Crystallisation may be complete as soon as the solution is cold, or it may take several hours, or days.

(iii) Collecting of Pure Crystals.

Any crusts of crystals which may have formed on the sides and edges of the vessel by evaporation must be removed before the remainder of the crystals are collected, as they are impure. They are carefully scraped off with a spatula and collected and returned to the mother liquor after filtration.

The filtration of crystalline compounds is best effected by means of a perforated porcelain plate placed in a funnel, or a complete funnel of porcelain having per-forations for this purpose (Buchner or Hirsch funnel). The perforations are covered over with a filter paper of the right size and to prevent break-ing of the paper two thick-nesses may be used, or har-dened filter paper. The paper is wetted with the liquid and sucked down by a vacuum produced by a filter pump (Fig. 9).
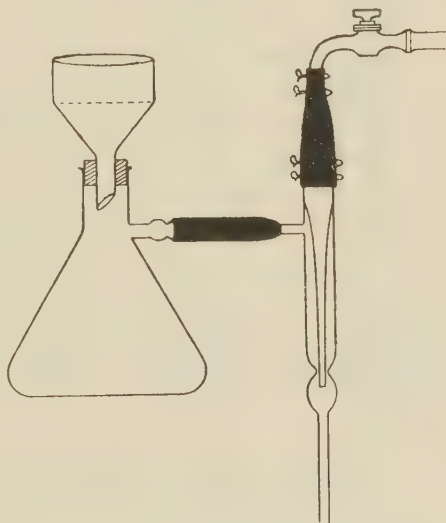
The solution and substance are poured upon the paper and the liquid drained off as com-pletely as possible, the solid

FIG. 9.

being pressed down with a spatula or flat piece of glass. The vessel is rinsed out with a little solvent which is used also for washing the crystals, and washing is done two or three times.

(iv) Drying of Pure Crystals.

The crystals are left to drain as completely as possible in the funnel and are then transferred to either

(1) several thicknesses of filter paper and the liquid pressed out ;

(2) a piece of unglazed porous plate, carefully dusted before use ;

(3) a watch or clock glass and dried in the air, or placed in a
    vacuum desiccator over sulphuric acid, soda lime, etc.

Sometimes the crystals, if placed on a watch glass and when they are
nearly free from solvent, are dried by putting the glass containing them
on a boiling water-bath. The crystals in this case should not melt
below 100° or contain sufficient solvent that they dissolve in it on
warming. They are cooled in a desiccator.

(v) Mother Liquor.

The mother liquor generally contains some dissolved solid, which
should be recovered. The impure crusts, if any, are added to the
liquid and the liquid is concentrated by distilling or evaporating (p. 11)
until crystals begin to separate. The solution is poured or filtered into
a beaker and allowed to cool; a second crop crystallises out and is
treated as above. A third and more crops may be obtained on further
concentration. These are not so pure as the original crop, but may
be recrystallised and obtained pure.

(vi) Decolorising Solutions.

Substances containing tarry or resinous impurities, or colouring
matter, cannot sometimes be freed from them by simple recrystallisation.
During recrystallisation and while the solid is in solution (especially
aqueous or alcoholic), the solution is boiled for 2-5 minutes or longer
with a small quantity of blood charcoal which is removed by filtration.
The first portions of filtrate generally require filtering again through
the same paper as the finely divided charcoal passes through at first.
To remove colouring matter from a solution which should be colour-
less, prolonged boiling with several quantities of charcoal is sometimes
necessary.

DETERMINATION OF THE MELTING-POINT.

A small quantity of the substance is introduced into a melting-
point tube; this is attached to a thermometer and the two are heated
together in a bath until the substance is seen to melt. The first de-
termination of an unknown substance is usually only approximate:
it is repeated, heating rapidly to within 10° and then more slowly.

A melting-point tube consists of a capillary of thin glass about
1 mm. in diameter, 5-6 cm. long and closed at one end. It is
made by heating near one end a dry piece of glass tubing of about
1 cm. bore in a blow-pipe flame until it is red-hot and soft, removing
it from the flame and pulling it out carefully just when the glass begins

to harden.   A long capillary tube is thus made.   Several more such lengths can be made from the glass tube if the capillary so made be broken off about 2-3 inches from the remainder of the glass tube. The long capillaries are cut into short lengths of about 5-6 cm. by scratching at these distances with a file and breaking by bending. Short lengths and lengths of too small bore must be rejected.   One end of each capillary is sealed by holding it in a small flame.   The tubes so made are preserved in a corked dry test tube.

The substance is finely powdered and introduced by scooping up solid with the open end and making it fall to the other end by gently tapping the closed end on the bench.   This process is repeated until sufficient of the substance to occupy a length of 2-5 mm. in the capillary has been introduced and shaken down, or pushed down with a fine wire, so as to form a compact and continuous layer.

The filled melting-point tube is attached to the thermometer so that the substance is on a level with the bulb. The attachment is made with a strip of rubber cut from a length of rubber tubing.

A small beaker containing water is used as a bath if the melting-point is below 100°, and the thermometer in a cork is held in the centre of it by a clamp.   The beaker is heated over a gauze by a small flame and the liquid is stirred with a circular glass stirrer.

A flask of about 50 c.c. capacity with a long neck (10-20 cm.) filled about two-thirds with strong sulphuric acid is more generally used.   The thermometer is secured in a cork into which a notch is cut

FIG. 10.

to allow hot air to escape when the flask is heated and to see the graduations if the mercury reaches this level.   The flask is held by a clamp and heated with a flame directly, the burner being inclined at an angle and held by the hand so that it is heated round and round and not directly in the centre (Fig. 10).   After frequent use the acid becomes dark in colour, but it will become clear again if a tiny crystal of potassium nitrate be added.

If a bath of sulphuric acid be used, the attachment of the melting-point tube is made by adhesion.   The thermometer is wetted with acid and if held horizontally the acid runs along it.   The melting-point tube is wetted with acid by drawing it along the wetted thermometer, and it
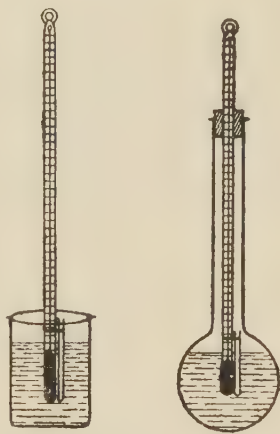
will adhere when the surfaces of contact are wet and will not fall off on putting it carefully into the bath.

Paraffin wax is used when the melting-point of a substance is above the boiling-point of sulphuric acid (290°). The solid wax is introduced and melted until it fills two-thirds of the space. It becomes brown after being used several times and must be renewed.

*   The pure specimens of benzoic acid and urea may be used for determination of the melting-point.

CHAPTER II.

A PURE carbon compound can now be analysed, i.e. the elements contained in it are ascertained and their amounts are determined.

Carbon compounds may contain the elements hydrogen, oxygen, nitrogen, halogens, sulphur, phosphorus, etc., either singly or collectively. Usually all the possible elements are not present at the same time, but the proteins contain carbon, hydrogen, oxygen, nitrogen, and sulphur; some contain also phosphorus and a few contain halogens. Proteins belong to the most complex of the organic compounds.

The elementary composition, or detection of the elements, precedes the quantitative composition. Since all organic compounds contain carbon and most of them contain hydrogen it is not absolutely essential that the presence of these elements should be ascertained. The quantitative analysis of the compound is varied according to the elements which are present.

A. ELEMENTARY COMPOSITION.

DETECTION OF THE ELEMENTS.

1. Carbon and Hydrogen.

(*a*) A small portion of the substance is gently heated in a *dry* test tube. It melts and chars. The charring denotes the presence of carbon. A condensation of water on the sides of the tube where it is cool denotes the presence of hydrogen. As this simple method does not show the presence of carbon and hydrogen in all compounds, it is in these cases necessary to proceed as follows :—

(*b*) About 5 grms. of finely powdered cupric oxide are dried thoroughly by heating in a small crucible. Whilst still warm[1] it is mixed with a little of the substance (e.g. oxalic acid) and the mixture is introduced into a hard glass tube. The end is closed with a cork through which passes a glass tube, bent at right angles. This end is dipped into a little baryta water contained in a small beaker or test

---

[1] As cupric oxide takes up water on cooling it must be used warm, otherwise it must be allowed to cool in a desiccator over sulphuric acid.

tube. On heating the mixture in the hard glass tube, water will condense on the cooler parts of the tube—presence of hydrogen; and the baryta water will become turbid owing to the formation of barium carbonate—presence of carbon.

2. Nitrogen.

(a) *As Ammonia.*—A portion of the substance is ground up with soda-lime and heated in a dry test tube. Ammonia is given off as shown by the smell, by litmus paper, and by the production of white fumes when a glass rod dipped in hydrochloric acid is held over the mouth of the tube (Will and Varrentrapp's method).

The peculiar smell of burning flesh, horn, etc., produced on heating such substances alone, also indicates the presence of nitrogen. The following method is preferable and is the most usual :—

(b) *As Sodium Cyanide.*—A small piece of metallic sodium is heated in a small dry test tube of hard glass until the metal begins to boil ; successive minute portions of the substance are added. It is important that the substance be made to come into proper contact with the sodium. The tube is heated to redness for a short time, cooled, and the lower end of it is broken in a mortar, containing a few drops of alcohol ; water is added when effervescence has ceased. The solution is transferred to a test tube, warmed and filtered. To the filtrate some ferrous sulphate solution (this must be freshly prepared by dissolving a few small crystals in a little water) and caustic soda are added and it is boiled for a few minutes. It is cooled and a drop or two of ferric chloride and excess of dilute hydrochloric acid are added. A precipitate or coloration of Prussian blue indicates the presence of nitrogen (Lassaigne's method).

The following reactions occur :—

$$\text{Na, C, N} \rightarrow \text{NaCN} ; \quad 2\text{NaOH} + \text{FeSO}_4 = \text{Fe(OH)}_2 + \text{Na}_2\text{SO}_4.$$
$$6\text{NaCN} + \text{Fe(OH)}_2 = \text{Na}_4\text{Fe(CN)}6 + 2\text{NaOH}.$$
$$3\text{Na}_4\text{Fe(CN)}6 + 4\text{FeCl}_3 = \text{Fe}_4\{\text{Fe(CN)}6\}_3 + 12\text{NaCl}.$$
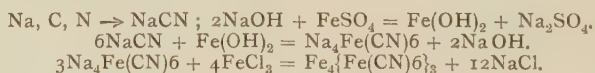
Castellana's Modification of this Test.—A small quantity of the substance is intimately mixed with about ten times its quantity of equal parts of magnesium powder and dry sodium carbonate and gently heated until the magnesium burns ; it is then heated to redness as with the sodium (b). The remainder of process is carried out as described above, i.e. breaking the tube in a mortar, etc.

3. Halogens.

(a) *Beilstein's Test.*—A piece of copper wire is heated in a Bunsen flame until the flame is no longer coloured green. A little of the sub-

stance, e.g. chloroform, is placed on it and it is again heated. Copper chloride is formed which colours the flame green. This test is not always satisfactory.

(b) *Halogen* is generally detected by means of sodium employed just as in the nitrogen test. The filtered solution is acidified with nitric acid, boiled to remove any hydrocyanic acid which will be formed if the substance also contains nitrogen, and then treated with silver nitrate.

(c) *Heating with Lime.*—Halogens are best detected by heating with quicklime. The substance is finely powdered and mixed intimately with lime (if liquid, e.g. chloroform, the lime is moistened with the substance) and then heated strongly. When cool, water is added and the lime dissolved in nitric acid. On adding silver nitrate, a precipitate of silver halide is obtained if halogen be present.

The nature of the halogen may be determined by treating a little of the acidified solution with chlorine water, and then testing with starch solution for iodine, or by extracting with carbon bisulphide or chloroform for bromine.

4. Sulphur.

(a) As sodium sulphide. A small portion of the substance is heated with metallic sodium as described under 2 (b). The hot tube is broken in a little water, the contents are filtered and tested for sodium sulphide with (1) lead acetate, (2) sodium nitroprusside.

(b) As sulphate. A small portion of the substance is fused in a crucible with three times its quantity of fusion mixture $\{2KNO_3 + Na_2CO_3\}$. The mixture is heated cautiously at first round the edge and the heating is continued after the fusion until all charred particles have vanished. The mass, when cool, is extracted with hot water and the filtered solution is tested for sulphates with barium chloride in the presence of mineral acid (HCl or HNO_3).

5. Phosphorus.

(a) Some caseinogen is fused with fusion mixture as described for sulphur, the fused mass is extracted with hot water, and the solution is divided into two parts. To the one part is added excess of nitric acid and ammonium molybdate: a yellow precipitate on warming indicates phosphoric acid; to the other part excess of ammonia is added and phosphates are precipitated with magnesia mixture.

(b) A small quantity of caseinogen in a small flask is covered with 5-10 c.c. of concentrated sulphuric acid and an equal volume of concentrated nitric acid is added. The mixture is heated gently over

a small flame (in the draught chamber) until the mixture becomes colourless. If it becomes brown, it is cooled, more nitric acid is added and it is heated again. When it is colourless, it is allowed to cool, water and a little ammonium nitrate solution are added and it is heated nearly to boiling; on adding ammonium molybdate solution, a yellow colour or precipitate indicates the presence of phosphoric acid (Neumann's method).

6. Other Elements.

Amongst natural compounds the most important other elements combined with carbon are iron, e.g. iron in hæmoglobin, ferrocyanides, and magnesium, e.g. magnesium in chlorophyll. Copper is found in certain other animal pigments. Silicon is present in certain vegetables, e.g. in grasses. Organic silicon, arsenic, antimony and magnesium compounds have been prepared in the laboratory. These elements are best detected after the organic matter has been completely removed by burning either alone or in the presence of an oxidising agent (fusion mixture). Thus :—

*   **Detection of Iron in Hæmoglobin.**—A small portion of hæmoglobin is heated in a crucible with 3-4 times its quantity of fusion mixture until all the organic matter has been oxidised. The mass, when cold, is dissolved in dilute hydrochloric acid. The solution is filtered and the filtrate is tested for ferric salts with (*a*) ammonium thiocyanate, and (*b*) potassium ferrocyanide.

B. QUANTITATIVE COMPOSITION.

ESTIMATION OF THE ELEMENTS.

1. Carbon and Hydrogen.

An organic compound on oxidation with copper oxide is converted into carbon dioxide and water. The amount of each element contained in the compound is determined by weighing the carbon dioxide and water produced from a known weight of the compound.

The analysis is carried out in a long tube of hard glass, a combustion tube, about 80 cm. long, which is heated in a furnace (Fig. 11). Five-eighths of the length of the tube is filled with coarse copper oxide which is kept in position by narrow plugs of oxidised copper gauze. Next to the copper oxide there is a small boat (copper or porcelain) of suitable size, containing a known weight of the substance, and the remaining space is filled by a roll of oxidised copper gauze (Fig. 12). This end of the tube is connected with a gasometer

containing air (or oxygen) and a current of air freed from carbon
dioxide and water by passing through potash and sulphuric acid or
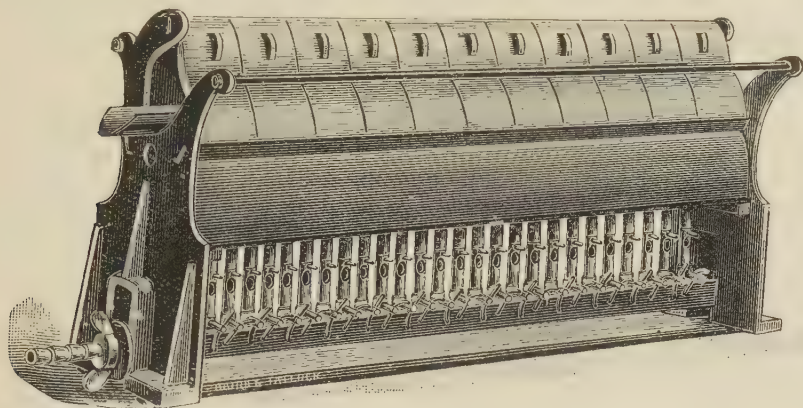
FIG. 11.—Combustion Furnace.

calcium chloride, is passed through the combustion tube so as to drive
out the products of the combustion and to help in the oxidation.
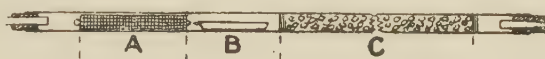
A = Oxidised Roll of Copper Gauze.
B = Boat.
C = Coarse Copper Oxide.

FIG. 12.—From Price and Twiss' "Practical Organic Chemistry."

To the other end of the combustion tube are attached absorption tubes
(Fig. 13), of which there are various forms, to collect the carbon dioxide
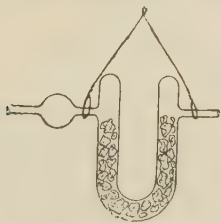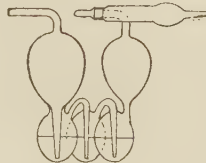
Calcium Chloride Tube.   Potash Absorption Bulbs.
FIG. 13.—From Price and Twiss' "Practical Organic Chemistry."

and water.   The first absorption tube, generally of U-shape, contains
calcium chloride [1] or pumice wetted with concentrated sulphuric acid ;
the second, generally complex in shape so as to give several surfaces,

[1] After filling the tube with calcium chloride dry carbon dioxide must be passed through
it until its weight remains constant.

contains caustic potash of 33 per cent. strength; a small tube containing calcium chloride is attached so as to retain water vapour, which may be carried away during the passage of the gases. These tubes are weighed before and after the combustion and their increase in weight gives the required data.

In practice, the combustion tube is filled, as described, with coarse copper oxide, which has been heated to redness in a copper basin and allowed to cool. The plugs and the roll are made of copper gauze which is rolled round a piece of copper wire and heated in a blow-pipe flame to oxidise the metallic copper and burn away any organic matter. A space is left for the boat. The tube is heated in the furnace at a low red heat and a current of dry clean air or oxygen is passed through the tube from the gasometer. The carbon dioxide and water present in the tube and on the copper oxide are thus removed and the copper is completely oxidised to copper oxide. The heating of that portion of the combustion tube into which the boat is to be placed is discontinued so that this portion cools to room temperature whilst the rest of the tube is kept at a red heat. The absorption tubes are filled and weighed. About ·2 gm. of substance is exactly weighed out into the boat which has been heated and cooled in a desiccator. When the end of the combustion tube is cool the absorption tubes are attached, that for water next to the tube. From the other end the roll is removed with a hooked copper wire, the boat quickly introduced and the roll replaced. The tube is closed and the pure air or oxygen current passed through at a rate of about 3 bubbles every two seconds. The roll is heated commencing at the end farthest from the boat, and the heating is gradually extended from this point towards the boat and the coarse copper oxide until the substance has burnt away and the whole tube is heated from end to end. The air or oxygen current is continued for about half an hour after the combustion is finished so as to drive out the water and carbon dioxide. Any water which condenses on the end of the combustion tube is driven into the absorption tubes by means of a small flame, or hot brick, held under the end of the combustion tube. When the oxidation is completed, the absorption tubes are removed, allowed to cool for $\frac{1}{2}$ to 1 hour and weighed.

This method of analysis requires some modification if elements other than hydrogen or oxygen are present in the substance.

(a) *Halogens.*—On combustion, the halogen in an organic compound is evolved as hydrogen halide, or as halogen. To prevent its entry into the absorption tubes it is combined with silver as silver halide. This is effected by putting at the end of the combustion tube a roll of silver gauze.

(b) *Nitrogen.*—Oxides of nitrogen may be evolved when organic nitrogenous compounds are analysed. A roll of metallic copper gauze, prepared by heating a roll in a blow-pipe flame and dropping it into a few c.c. of methyl alcohol contained in a test tube (held in a duster) and drying at 100°, is introduced at the end of the combustion tube. Any oxides of nitrogen are thus reduced to nitrogen and prevented from being absorbed by the potash.

(c) *Sulphur and Phosphorus.*—To prevent hydrogen sulphide or hydrogen phosphide being formed, the oxidation of the organic compound is effected with lead chromate instead of copper oxide and the substance is mixed with it instead of being placed in the boat. Lead chromate may replace the coarse copper oxide entirely or about half of it.

Organic phosphorus compounds are very difficult to oxidise completely,

and frequently give results for carbon which are too low by about ·5 to 1 per cent.

2. Nitrogen.

(a) **Dumas' Method.**—On heating an organic compound containing nitrogen with copper oxide, its nitrogen is given off as nitrogen. The gas given off from a known weight of substance is collected and its volume measured, from which value its weight can be calculated.

The analysis in practice is carried out in a similar way to that described for carbon and hydrogen, but the substance is mixed with finely powdered copper oxide and introduced into the tube, and a roll of reduced copper gauze is placed at the end of the combustion tube so as to reduce any oxides of nitrogen, which may be formed, to

FIG. 14.

nitrogen. Instead of a current of air, a current of carbon dioxide is passed through the tube. The gas is collected in a Schiff's nitrometer over caustic potash which absorbs the carbon dioxide leaving the nitrogen (Fig. 14).

There are several ways of passing the carbon dioxide through the combustion tube ; it may be evolved from a Kipp apparatus or it may be evolved by heating magnesite, either contained in a special tube or in the combustion tube, which in this case is sealed at the end. It is obvious that before carrying out the analysis all the air must be expelled from the apparatus.

(b) **Kjeldahl's Method.**—The principle of this method consists in oxidising the substance with concentrated sulphuric acid ; the nitrogen

is converted into ammonia.   The solution is made alkaline with caustic soda and distilled.   The ammonia is evolved and is collected in excess of standard acid; on subsequent titration with standard alkali the amount evolved is given by difference.

This method is much simpler to carry out than the Dumas' method, but it cannot be employed for all nitrogen-containing compounds.

On account of its simplicity this method has found extensive use in biological chemistry.   The large group of compounds—the proteins—all contain nitrogen; the amount of protein in a solution is estimated by determining the nitrogen content (see below); and the amount of nitrogen in urine is a factor of importance in studying metabolism.

In most laboratories there is an apparatus in which six determinations can be carried out at the same time as in Fig. 16, p. 28.

*   Example: Estimation of Nitrogen in Egg-white Solution.

A known weight, say 0·3 to 0·5 gm., or a known volume, say 5 c.c., of an egg-white solution is placed with a pipette into a clean round-bottom Jena glass flask of 700 c.c. capacity.   10 or 20 c.c. of pure concentrated sulphuric acid and a crystal of copper sulphate, about the size of a pea and weighing about 0·25 gm., which helps in the oxidation, are added.   (1 gm. of potassium sulphate is also sometimes added for this purpose, as it raises the temperature.)   The flask is heated in a fume-cupboard until the liquid, which at first becomes brown from charring, becomes quite or nearly colourless, a process which takes about an hour.   The flask is allowed to cool and is half-filled with distilled water.   By means of a special distillation tube it is connected to a condenser set up in a vertical position as in Fig. 15, No. 1.

With a pipette a quantity of standard sulphuric acid (5 c.c. of N or 50 c.c. ·1 N H_2SO_4) are measured out into a clean beaker or flask of about 600 c.c. capacity, and this is placed below the condenser so that the end of the condenser just reaches the surface of the liquid. It is preferable to add a few drops of indicator, methyl orange or alizarin red, before the distillation is commenced, in case more acid is required than that originally taken; the change in colour of the indicator gives notice of this fact.

The round-bottomed flask is removed, a piece of porous earthenware is added, and excess of caustic soda solution (40 c.c. of 40 per cent. for every 10 c.c. concentrated H_2SO_4 used) is run in under the dilute acid without mixing.   The flask is connected again to the condenser

seeing that all corks fit tightly.   The soda and acid are mixed and the ammonia is distilled off into the acid.   In about half an hour the ammonia will have completely passed over into the standard acid.   To test if the distillation is finished the flask is lowered and the condensed water is allowed to wash out the inside of the condenser tube for about two minutes : after this time the distillate is tested with litmus paper to ascertain if any more ammonia is being evolved.   When finished,

No. 1.   No. 2.
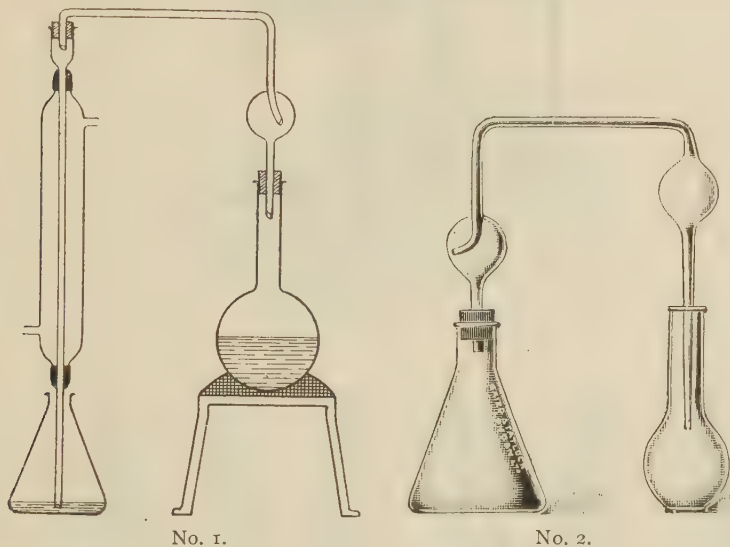
FIG. 15.

the outside of the condenser tube is washed with distilled water and the contents of the flask are titrated with ·1 N alkali.

Sometimes the distillation is carried out without using a condenser (Fig. 15, No. 2); the end of the special distilling tube is then dipped into the standard acid.   There is in this case usually greater danger of the liquid being sucked back into the flask which is being distilled, and further the glass is attacked by the ammonia with liberation of alkali, which causes inaccuracies in the determination.

The difference between the amount of standard alkali and standard acid gives the amount of ammonia evolved, from which the amount of nitrogen can be calculated :—

$$5 \text{ c.c. N } H_2SO_4 = 50 \text{ c.c. ·1N } H_2SO_4.$$
$$37\text{·}4 \text{ c.c. ·1N NaOH used.}$$
$$\overline{12\text{·}6 \text{ c.c. ·1N difference.}}$$

$$12\text{·}6 \text{ c.c. ·1N } H_2SO_4 = 12\text{·}6 \text{ c.c. ·1N } NH_3$$
$$= 12\text{·}6 \text{ c.c. ·1N Nitrogen.}$$
$$= 12\text{·}6 \times 0\text{·}0014 \text{ gm. Nitrogen.}$$

∴   5 c.c. solution contain 0·01764   ,,   ,,
∴ 100 c.c.   ,,   ,,   0·3528   ,,   ,,
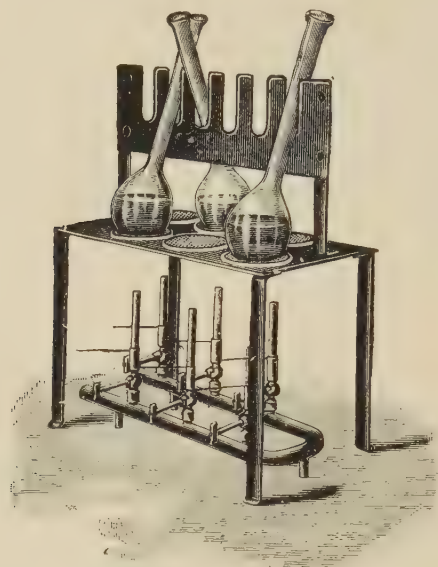
No. 1.
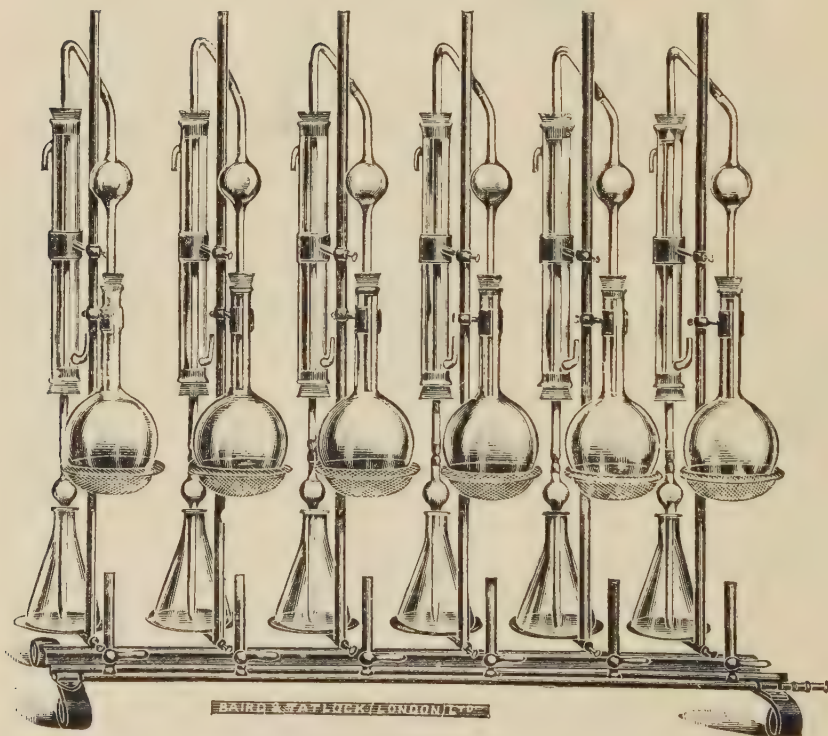
No. 2.

FIG. 16.—Apparatus for estimation of nitrogen by Kjeldahl's method.

The amount of protein to which this amount of nitrogen corresponds is ascertained as follows :—

Proteins contain from 15 to 16 per cent. of nitrogen.   Taking 15·5 as the average value, $\dfrac{100}{15\cdot5}$ or 6·45 times the amount of nitrogen gives the amount of protein.   The factor 6·25 is, however, generally used.

100 c.c. solution thus contain 6·25 × 0·3528 gm. protein = 2·2 gm.

3. Halogens.

After completely oxidising all the organic matter to carbon dioxide and water, the halogens are present as inorganic compounds and are estimated in the usual way.

(*a*) The commonest method is to oxidise a known weight of the substance in a sealed tube with fuming nitric acid at 200° for several hours, a few crystals of silver nitrate being at the same time placed in the tube : silver halide is formed and this is washed out of the tube and weighed (Carius).

In practice, about ·2 gm. of the substance is weighed out into a narrow test tube about 2 in. long.   Some silver nitrate crystals are placed in a thick-walled combustion tube sealed at one end and covered with 10-20 c.c. of concentrated nitric acid, care being taken not to wet the sides of the tube with acid.   This is done by introducing the acid through a tube with a long capillary. The test tube containing the substance is put in avoiding contact of the substance with the acid.   The open end of the tube is now sealed in the following way : a glass rod for a handle is fastened by heating to the side of the tube at the open end.   The tube is heated near this end in a blow-pipe flame in such a way that the walls collapse together; when it is nearly closed the end is drawn out so as to form a capillary tube with even and thick walls.   The capillary is sealed by pulling off the end with the handle attached.   The tube is wrapped in asbestos paper and carefully placed, capillary outwards, in an iron tube which can be closed with a screw cap.   The iron jacket and tube are placed in a specially constructed furnace in such a position that the capillary point faces a wall.   Should the tube burst, the contents are then not blown into the room.   The tube is heated to about 200-220° for 4 or 5 hours.   The furnace and contents are allowed to cool.   The cap of the iron tube is removed and the capillary point allowed to project a little.   The point is heated in a flame.   When the glass is soft the pressure inside the tube forces an opening.   Owing to the high pressure inside the tube it is unsafe to open the tube in any other way.   The capillary is cut off and the contents washed out into a beaker.   The silver halide is filtered off and weighed by the usual method employed in inorganic chemistry.

(*b*) Less frequently, halogens are estimated by heating the substance in a combustion tube with quicklime.

A thin layer of quicklime is placed at the closed end of a combustion tube. Next to this is put a mixture of the substance (about ·2 gm.) with quicklime and then another layer of quicklime. The tube is heated in a furnace, as in the estimation of carbon and hydrogen, the layers of quicklime being heated to redness before the mixture of substance and lime.

The contents of the tube, after the oxidation, are dissolved in nitric acid and the halogen precipitated with silver nitrate. The silver halide is filtered off, washed, dried, and weighed.

(c) A convenient method of estimating chlorine is that described by Neumann. The substance is oxidised with a mixture of nitric acid and sulphuric acid. Hydrochloric acid is evolved, and this is collected in a solution of silver nitrate. After boiling the solution for about half an hour to remove hydrogen cyanide, which is also formed if nitrogen be present in the substance, the silver chloride is filtered off, washed, dried, and weighed.[1]

4. Sulphur.

This element is most generally estimated by the same method as the halogens (Carius); sulphuric acid is formed and precipitated as barium sulphate.

It is more convenient to oxidise the substance in a nickel crucible with sodium or barium peroxide ; the contents are acidified with hydrochloric acid and the barium sulphate formed is weighed. Still more convenient is the oxidation mixture used for estimating total sulphur in urine (see under urine).

5. Phosphorus.

Phosphorus is usually estimated in the same way as sulphur by the Carius method, the phosphoric acid formed being precipitated as ammonium magnesium phosphate.

The most rapid and convenient method is that of Neumann. The substance is oxidised in an open flask with a mixture of nitric and sulphuric acids. The phosphoric acid formed is precipitated as ammonium phosphomolybdate and this is then estimated by solution in excess of ·5N caustic soda and subsequent titration with ·5N sulphuric acid. The difference between ·5N NaOH and ·5N H_2SO_4 multiplied by 1·268 gives the number of milligrams of P_2O_5 in the given weight of substance taken.[2]

C. CALCULATION OF RESULTS.

With the exception of oxygen all the elements present in an organic compound are thus estimated. The amount of oxygen is found by difference.

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[1] See Plimmer, J. Physiol., vol. 31, p. 65.
[2] See Plimmer and Bayliss, J. Physiol., 1906, 33, 439.

From the figures obtained the percentage composition is calculated, i.e. the amount given by 100 gm. of substance, thus :—

0·2009 gm. substance gave 0·2987 gm. CO_2 and 0·1092 gm. H_2O.
0·1887 gm. ,, ,, 15·2 c.c. moist N at 16·5° and 767 mm.

Now, 0·2987 gm. CO_2 = 0·2987 × $\frac{12}{44}$ gm. C = 0·2987 × $\frac{3}{11}$ gm. C = 0·0815 gm. C.

0·1092 gm. H_2O = 0·1092 × $\frac{2}{18}$ gm. H = 0·1092 × $\frac{1}{9}$ gm. H = 0·01213 gm. H.

15·2 c.c. moist N at 767 mm. and 16·5° C. = $\frac{15·2 \times 753^* \times 273}{760 \times 289·5}$ c.c. at 0° and 760 mm.

$$= 14·2 \text{ c.c.}$$
$$= 14·2 \times \frac{28}{22,400} \text{ gm.}$$
$$= 0·01775 \text{ gm. N.}$$
$$\therefore \text{ percentage of C} = \frac{0·0815 \times 100}{0·2009} = 40·56.$$
$$H = \frac{0·01213 \times 100}{0·2009} = 6·04.$$
$$N = \frac{0·01775 \times 100}{0·1887} = 9·40.$$
$$O \text{ by difference} = \underline{44·00.}$$
$$\text{Total} = \overline{100·00}$$

The formula of the compound is obtained by dividing the percentages by the atomic weights of the elements ; the ratio of the number of atoms to each other is then obtained by dividing by the lowest value :—

$$C \frac{40·56}{12} = 3·38 \div 0·67 = 5.$$
$$H \frac{6·04}{1} = 6·04 \div 0·6, = 9.$$
$$N \frac{9·40}{14} = 0·67 \div 0·67 = 1.$$
$$O \frac{44·0}{16} = 2·75 \div 0·67 = 4·1.$$

The formula of the compound is therefore $C_5H_9NO_4$.

In any estimation only a difference of 0·2–0·3 per cent. is allowed between the values found and those calculated from the formula. The calculated values are

C = 40·81.   diff. = − 0·25.
H = 6·12.   = + 0·08.
N = 9·52.   = − 0·12.

The analysis was therefore sufficiently accurate.

D. DETERMINATION OF THE MOLECULAR WEIGHT.

As will be seen later, several organic compounds can have the same empirical formula, thus, for instance, lactic acid $C_3H_6O_3$ and glucose $C_6H_{12}O_6$ have the same empirical formula, namely CH_2O.

* Vapour pressure of water at 16·5° C. = 14·0 mm. ∴ pressure on gas = 767 − 14 = 753 mm.

In order to ascertain which of these formulæ is the correct one, a molecular weight determination is carried out, i.e. the weight of the molecule of the substance compared with that of a molecule of hydrogen (Avogadro's law).

The methods employed to determine the molecular weight are of two kinds : (*a*) physical, (*b*) chemical.

(*a*) Physical Methods.

1. **Victor Meyer's Method.**—Of the physical methods, that by Victor Meyer is the most frequently used when the substance can be vaporised without decomposition. A known weight of the substance is converted into vapour at a temperature 40-50° above its boiling-point in a special apparatus. The air previously contained in the apparatus is displaced by the vapour, collected in a graduated cylinder, and its volume measured ; this volume, after making corrections for temperature and pressure, corresponds to that occupied by the substance.
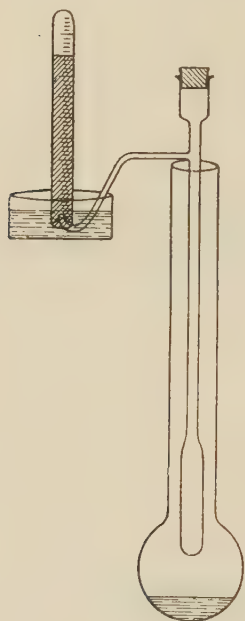
FIG. 17.

Thus, if v c.c. are given by w grammes substance,

$$\therefore 22{,}400 \text{ c.c. are given by } \frac{w \times 22{,}400}{v}$$

$$= \text{mol. wt.}$$

The apparatus employed is shown in the accompanying Fig. 17. A liquid, boiling 40-45° above the temperature at which the substance is volatilised, is boiled in the round bulb of the outer vessel. As soon as the temperature is constant and no more air escapes from the inner vessel by the side tube, the inverted graduated cylinder, filled with water, is placed over the end of the side tube, the cork is removed and a known weight of substance, contained in a small glass vessel, is dropped through the opening into the inner vessel and the cork is quickly replaced. The substance is rapidly vaporised and the vapour displaces an equal volume of air, which is driven out and collected and measured in the graduated cylinder.

2. **Raoult-Beckmann Method.**—Substances dissolved in a liquid lower its freezing-point. It was shown by Raoult that the freezing-point was lowered the same number of degrees when weights of different substances proportional to their molecular weights were dis-

solved in the same volume of liquid. Each liquid was found to have a definite freezing-point. By employing this value as a constant, the molecular weight of an unknown substance can be found. It is given by the formula,

$$M = \frac{100 \times C \times w}{d W},$$

where C is the constant, w the weight of the substance, W the weight of the solvent, and d the depression of the freezing-point.

The constants are :—water 19   benzene 49

acetic acid 39   phenol 76

Conversely by determining the lowering of the freezing-point, the amount of salt in a solution can be ascertained, e.g. in serum, urine.

The apparatus (Fig. 18) devised by Beckmann consists of the freezing-point tube C with side opening D. It is closed by a cork through which a Beckmann thermometer T and a stirrer S (through a glass tube) pass. The Beckman thermometer is a large thermometer graduated usually in $\frac{1}{1000}$ parts of a degree and having a range of only 5-6°.[1] The tube C is placed in a wider .tube B which serves as a jacket and prevents too rapid cooling. This is fixed in position in a freezing mixture of salt and ice in the vessel A by a cork which fits the opening in the brass lid L. The brass lid has also openings for the passage of a stirrer E and a thermometer. In carrying out a determination a known weight of solvent is placed in C and its freezing-point is taken. The tube is then removed and the solid allowed to melt. A known weight of substance is then introduced through D, dissolved in the liquid, and the freezing-point again determined.

Several determinations of the freezing-point of the solvent and the solvent containing the substance should be taken. Whilst the freezing-point is being taken the liquid becomes super-cooled. To prevent very great super-cooling it is vigorously stirred with the stirrer. At the freezing-point the temperature rises and the highest point reached is taken as the freezing-point.
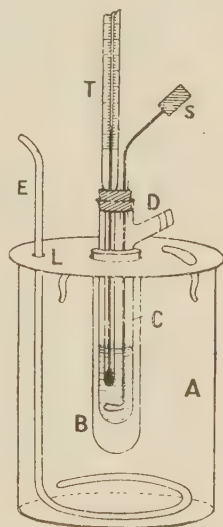
Fig. 18.—(From Findlay's " Practical Physical Chemistry.")

Similarly, a rise in the boiling-point of a solvent, when substances are dissolved in it, will give the molecular weight of the substance.

(b) Chemical Methods.

When the substance is an acid, or a base, the molecular weight can be determined by chemical methods.

[1] It is so constructed that mercury can be removed from the thread or introduced into the thread from a small bulb at the top. It can thus be used for any liquid.

(1) In the case of an acid :—

The molecular weight can be calculated from the amount of standard alkali required to neutralise, using phenolphthalein as indicator, a known weight of the acid, according to the equation :—

$$H \text{ acid} + NaOH = H_2O + Na \text{ acid},$$

e.g. x c.c. ·1N NaOH $= y$ gm. of acid.
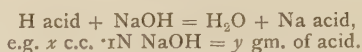
$$\therefore 40 \text{ gm. NaOH} = \frac{40 \times y}{x \times 0\cdot004}$$

$$= \text{mol. wt. of acid.}$$

If the acid be dibasic or tribasic, two or three molecules of sodium hydroxide will be required. The presence of such an acid will be indicated by titrating the acid using methyl orange, or alizarin red, and phenolphthalein as indicators. The acid salt will be neutral to methyl orange or alizarin red, the neutral salt to phenolphthalein. The basicity of the acid is definitely ascertained by the analysis of the salt and the free acid.

It is most usual to employ the silver salt of an acid. A quantity of the salt is prepared by adding silver nitrate to the neutral solution of the acid, filtering off the silver salt, washing and drying it. A known weight is heated in a crucible and the metallic silver obtained is weighed.

a gm. of silver salt gave b gm. of silver.

If the acid be monobasic, it will contain 1 atom of silver,

107·9 gm. of silver will be contained in $\dfrac{107\cdot9 \times a}{b}$ gm. of silver salt.

Since the silver replaces 1 atom of hydrogen,

$$\therefore \frac{107\cdot9 \times a}{b} - 107\cdot9 + 1 \text{ is the mol. wt. of the acid.}$$

If the acid be dibasic, it will contain 2 atoms of silver,

107·9 × 2 gm. of silver will be contained in $\dfrac{107\cdot9 \times 2 \times a}{b}$ gm. of silver salt.

Since the silver replaces 2 atoms of hydrogen,

$$\therefore \frac{107\cdot9 \times 2 \times a}{b} - (107\cdot9 \times 2) + 2 \text{ is the mol. wt. of the acid.}$$

The zinc salt or barium salt is also sometimes employed ; a known weight of salt is heated with a drop of concentrated sulphuric acid in a crucible ; zinc or barium sulphate is obtained from which the amount of barium or zinc is calculated.

(2) In the case of a base :—

Organic bases form double salts with metallic salts, such as platinum chloride, mercuric chloride. On heating the double salt in a crucible, a residue of the metal is left. The estimation of the amount of metal in a known weight of the compound gives the molecular weight.

Ammonia and platinum chloride give the compound ammonium platinum chloride,

$$(NH_3 . HCl)_2 . PtCl_4.$$

The organic bases form analogous compounds, the base replacing the ammonia.   Their general formula is therefore

$$(B . HCl)_2 . PtCl_4 \text{ or } B_2 . H_2PtCl_6.$$

The molecular weight of 2 molecules of base is thus

$$B_2 . H_2PtCl_6 - H_2PtCl_6,$$

e.g. x gm. of salt gave y gm. of platinum.
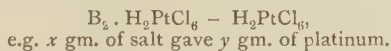
$\therefore$ 194·8 gm. platinum will be given by $\dfrac{194·8 \times x}{y}$ gm. salt $B_2 . H_2PtCl_6$.

Now 194·8 gm. platinum are contained in 409·8 gm. H_2PtCl_6.

$\therefore \dfrac{194·8 \times x}{y} - 409·8$ is the molecular weight of B_2.

Hence molecular weight of 1 molecule of base is $\dfrac{\dfrac{194·8 \times x}{y} - 409·8}{2}$

The gold salts have the general formula,

$$B . HCl . AuCl_3 \text{ or } BHAuCl_4,$$

from which the molecular weight of the base is calculated in a similar way.

IDENTIFICATION OF AN ORGANIC COMPOUND.

Knowing the formula of a pure organic compound from its analysis and molecular weight, it has to be identified.   The compound may be a known or an unknown one.

To find out if the compound is known reference is made to Richter's " Lexicon of Carbon Compounds "[1] in which the melting-points and other constants of the various compounds are given.   Corresponding properties identify the substance.

If the compound be unknown, further analysis is necessary ; it must be ascertained to what group of carbon compounds the unknown body belongs, whether it is an alcohol, an ester, an acid, a carbo-hydrate, an amide, an amine, a protein, etc.   With the complex natural substances this is a matter of great difficulty, and it may take many years before a question is settled ; e.g. tyrosine was discovered in 1846 and its constitution only definitely proved in 1882.

The identification of an unknown substance, or the rapid identifica-tion of a known substance, is greatly facilitated by a few preliminary tests.   If in solution, a portion should be evaporated to see if there is a residue and whether it is solid or liquid.   The residue can be tested for the elements present, especially nitrogen.   If there is no residue, the solution must be distilled and the boiling-point observed.

1. **Colour.**   Vegetable colouring matters : if blue, they are changed to red by acid and the blue colour is restored, or changed to a green, by ammonia ; if yellow, they are changed to brown by alkali and the colour is restored by acid.

Ferric salts and copper salts are reddish-brown and blue or green respectively.

Many coloured compounds show absorption spectra, such as hæmoglobin and its derivatives.

2. **Taste.**   Tasting must be done carefully on account of the extremely poisonous nature of some organic compounds.   A drop of a weak solution in water or alcohol may be used.   Acids have a sour and astringent taste.   Alkaloids and glucosides are bitter.   Sugars and glycerol are sweet.

3. **Odour.**   The odour is sometimes characteristic.

4. **Appearance.**   The appearance under the microscope gives evi-dence of homogeneity or impurity.   The microscopical appearance is very useful in identifying the different kinds of starch.   Many substances

[1] The most recent compounds are given in the yearly volumes of the Journals of the English and Foreign Chemical Societies.

have a characteristic crystalline structure, e.g. cholesterol, cystine, osazones of carbohydrates, etc.

5. **Effect of Heat.** By heating the substance, firstly on platinum, secondly in a small dry tube, many valuable details can be ascertained. The odour may be peculiar, the substance may melt, char, decompose, sublime or boil. The melting-point and boiling-point of solids and liquids can be observed directly after such a preliminary examination.

6. **Detection of the Elements.** By ascertaining whether the substance does or does not contain nitrogen, it may be placed in either of the following groups :—

Non-nitrogenous.	Nitrogenous.
Hydrocarbons.	Cyanogen compounds.
Alcohols, phenols.	Amides.
Esters, ethers.	Amines.
Aldehydes.	Amino acids.
Ketones.	Guanidine compounds.
Acids and salts.	Purines.
Fats and cholesterol.	Proteins.
Carbohydrates.	

7. **Solubility.**

(*a*) Alkali salts and salts of bases,

The lower alcohols, aldehydes, acids, ketones, amides, amines

The polyhydric alcohols and carbohydrates

Phenols and hydroxy acids

In general, compounds containing several OH groups

} are soluble in cold water.

(*b*) Aromatic acids are insoluble or very slightly soluble, but dissolve in boiling water.

Starch is insoluble, and gives an opalescent solution with hot water.

Tyrosine, cystine and uric acid are soluble with difficulty in water.

Fats, higher fatty acids, and cholesterol are insoluble in water, but soluble in ether.

8. **Behaviour towards Reagents.**

(*a*) *Reaction of aqueous solution to litmus.*

A marked acid reaction indicates an acid or a phenol ; if there is an odour, it may be a volatile fatty acid or a phenol.

A neutral reaction indicates a salt of an acid or a base; an alcohol, aldehyde, ketone (note smell), carbohydrate. An ester in alcoholic or ethereal solution has also a neutral reaction.

An alkaline reaction indicates a base, or an acid dissolved in excess of alkali.

(*b*) *Sodium carbonate :* acids insoluble in water, e.g. uric acid, also cystine, tyrosine dissolve; bases insoluble in water do not dissolve, or if in solution are precipitated.

(*c*) *Sodium hydroxide :* ammonium salts are decomposed with evolution of ammonia and bases are liberated from their salts. On boiling, amides are decomposed, and esters are hydrolysed.

(*d*) *Hydrochloric acid :* acids insoluble in water do not dissolve or if in solution are precipitated; bases insoluble in water dissolve, e.g. tyrosine, cystine, aniline.

(*e*) *Sulphuric acid.*

(*f*) *Nitric acid.*

(*g*) *Bromine water.*

(*h*) *Permanganate.*

(*i*) *Effect of heating with soda lime.*

The exactly neutral solution may be tested with

(*j*) *Schiff's reagent*—for aldehydes.

(*k*) *Ammoniacal silver nitrate*—for aldehydes, reducing carbohydrates, etc.

(*l*) *Fehling's solution*—for aldehydes, reducing carbohydrates, etc.

(*m*) *Ferric chloride*—for phenols (violet or green colour),
for acetoacetic acid (claret colour),
for formates, acetates (reddish-brown colour, precipitate on boiling),
for lactates, oxalates (yellow colour).

(*n*) *Calcium chloride*—for oxalates, urates, etc. (insoluble calcium salts are precipitated).

(*o*) *Sodium nitroprusside and sodium hydroxide :*—

Acetone gives a red   colour, changing to purple with acetic acid.
Creatinine ,,   ,,   ,,   ,,   ,, yellow   ,,   ,,   ,,
Indole   ,, a blue-violet ,,   ,,   ,, blue   ,,   .,   .,

Confirmatory tests must be made after an indication of the nature of the substance has been obtained, according to the reactions given under the various groups of compounds.

CHAPTER III.

THE simplest organic compounds are the hydrocarbons, which consist of carbon united with hydrogen.

Analysis and molecular weight determinations have shown them to possess the following empirical formulæ. They can be arranged in several groups according to their properties, thus

Saturated Hydrocarbons.

CH_4, C_2H_6, C_3H_8, C_4H_{10}, C_5H_{12}, C_6H_{14} . . . $C_{20}H_{42}$, etc.

Unsaturated Hydrocarbons: Ethylene Series.

C_2H_4, C_3H_6, C_4H_8, C_5H_{10}, C_6H_{12}, etc.

Unsaturated Hydrocarbons: Acetylene Series.

C_2H_2, C_3H_4, C_4H_6, C_5H_8, C_6H_{10}, etc.

Aromatic Hydrocarbons.

C_6H_6, C_7H_8, C_8H_{10}, etc.; $C_{10}H_8$, $C_{14}H_{10}$, etc.

The existence of so many compounds depends on the fact that carbon is a tetravalent element, and that, unlike other atoms, the carbon atom can combine with itself many times, thus 2, 3, 4, 5, 6, etc., carbon atoms can be combined together.

The valencies of the carbon atom are usually represented by four lines or bonds, or by dots, symmetrically placed around the letter C. The carbon atom must, however, be considered as possessing three dimensions. The only figure which properly represents a carbon atom is that of a regular tetrahedron. The four bonds, or valencies, pass from the centre to the four angles, or corners; they are equal in length and equally placed at an angle of 109° 28'.
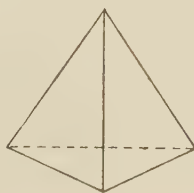
Represented on a plane, the combination of several carbon atoms appears in the form of a straight chain :—

$$-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}- \qquad -\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}- \qquad -\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-$$
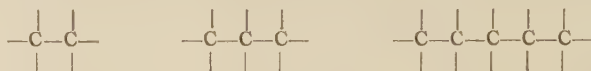
Actually, the carbon atoms are joined to one another at a corner, and if 5 or 6 carbon atoms be in a molecule there is close approach to ring formation.   A closed ring, such as occurs in benzene and aromatic compounds, is thus easily formed.   The same model for a carbon atom also gives a representation of the structure of unsaturated compounds.   For convenience, the formulæ of organic compounds is usually represented on a plane surface.   The tetrahedral model is required chiefly for compounds having optical activity (see under stereoisomerism).

A. SATURATED.

The saturated hydrocarbons are the compounds in which the remaining valencies of the carbon atoms joined together, as shown above, are satisfied by hydrogen :—

$$\begin{array}{ccc} H & H\ \ H & H\ \ H\ \ H \\ | & |\ \ \ | & |\ \ \ |\ \ \ | \\ H-C-H & H-C-C-H & H-C-C-C-H \\ | & |\ \ \ | & |\ \ \ |\ \ \ | \\ H & H\ \ H & H\ \ H\ \ H \end{array}$$

Methane.   Ethane.   Propane.
CH_4. CH_3-CH_3 $CH_3-CH_2-CH_3$
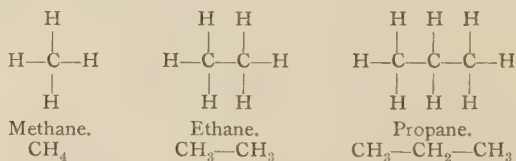
They form a homologous series of compounds in which the member containing 1 carbon atom more than the preceding one also contains 2 hydrogen atoms more, i.e. the members differ from one another by CH_2.   They possess the general formula C_nH_{2n+2}.

If we continue the process of adding CH_2 to propane, two ways are possible : it may be added to one of the end carbon atoms, or to the middle carbon atom.   The two compounds

$$\begin{array}{c} H \\ | \\ H-C-H \\ \ \ \ \ \ | \end{array}$$

$$\begin{array}{ccc} H\ \ H\ \ H\ \ H & H\ \ \ \ \ \ \ \ \ H & CH_3 \\ |\ \ \ |\ \ \ |\ \ \ | & |\ \ \ \ \ \ \ \ \ | & | \\ H-C-C-C-C-H \quad\text{and}\quad & H-C\ \ \ \ C\ \ \ \ C-H \ \ \text{or}\ \ CH_3-C-CH_3 \ \ \text{or} & \\ |\ \ \ |\ \ \ |\ \ \ | & |\ \ \ |\ \ \ | & | \\ H\ \ H\ \ H\ \ H & H\ \ H\ \ H & H \end{array}$$

$$CH_3{\Large\diagdown}\atop{CH_3{\Large\diagup}}CH-CH_3$$

$CH_3-CH_2-CH_2-CH_3$
Butane.

Isobutane or trimethyl-methane.
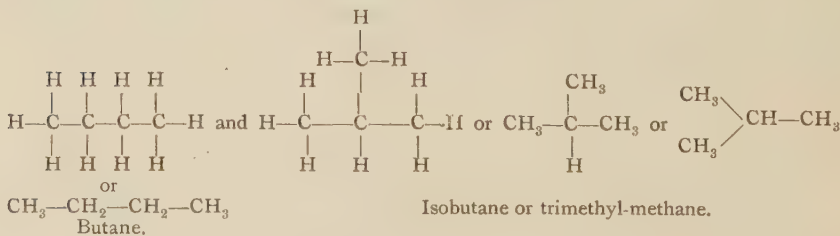
are thus obtainable.

Continuing the process of adding CH_2 to each of these hydrocarbons we obtain

Pentane.

Dimethyl-ethyl-methane.

Dimethyl-ethyl-methane.

Tetramethyl-methane.
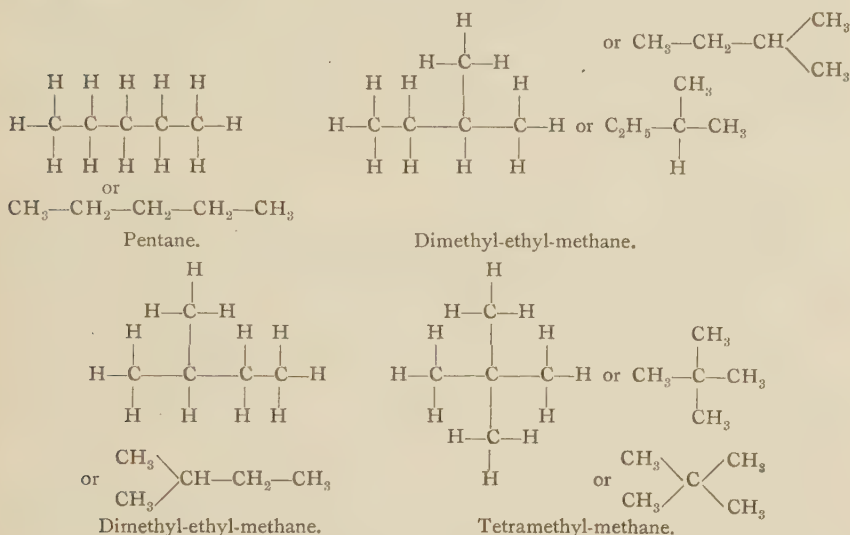
Two of these compounds are identical in structure, so that only three compounds containing five atoms of carbon can be derived from butane and isobutane.

Two compounds of the empirical composition, C_4H_{10}, and only two, are known. Three compounds of the formula C_5H_{12}, and three only, are known. The facts correspond with the theoretical explanation, which gives a structural, or graphic, formula to each hydrocarbon. The extension of the theory to hydrocarbons with 6 and more carbon atoms in their molecule shows how an enormous number of hydrocarbons are possible. The theory was advanced and developed to account for their large number. Proof of the structure is given by their method of preparation (No. 4, p. 44).

Two or more compounds which have the same empirical composition (C_4H_{10} or C_5H_{12}), but a different structure as represented by the graphic formulæ, are known as *isomers*. The phenomenon is called *isomerism*.

The compounds with a straight chain of carbon atoms, such as butane and pentane, are termed normal compounds. Those with a branched chain of carbon atoms are regarded as derivatives of methane, the radicles CH_3, C_2H_5, C_3H_7, etc., being termed methyl, ethyl, propyl, etc., which shows their origin from the parent hydrocarbon.

The saturated hydrocarbons are the basis of the nomenclature and classification of all the carbon compounds. The carbon compounds

are classified according as to whether they contain 1, 2, 3, or 4, etc., carbon atoms in their molecule, i.e., whether they are derived from methane, ethane, propane, butane, etc.

The saturated hydrocarbons are distinguished by the suffix *ane ;* the prefix *meth* means 1 carbon atom ; *eth* means 2 carbon atoms ; *prop* means three carbon atoms, and so on.

Occurrence.

The majority of the saturated hydrocarbons are natural substances. The lower members of the series of the hydrocarbons (up to 4 carbon atoms, which are gases) are formed by the dry distillation of diverse organic substances and are contained in coal gas. Methane occurs in coal seams, but was originally called marsh gas, because it was found to escape from the water of ponds, where it is now known to be formed by the decomposition of cellulose. By a similar process of bacterial action it may be produced in the intestines of animals.

The middle members, containing 5-16 atoms of carbon, are liquids, and are contained in petroleum, which consists of a mixture of saturated hydrocarbons. The higher members are solids.

Two theories have been advanced to account for their formation. According to the first, they are the products of the dry distillation of animal remains (fats) ; according to the second, they are formed by the action of water upon the metallic carbides, of which the interior of the earth is supposed to consist. If the former supposition be the correct one, as the most recent work tends to show, they become of still greater interest in biological chemistry.

Several fractions are separated by the fractional distillation of the natural mineral oil. The following are the principal fractions from American petroleum :—

1. Cymogene, B.P. 0°⎫ gases which are liquefied by pressure and used for producing
2. Rhigolene, B.P. 18°⎭ cold by evaporation.
3. Petroleum ether or naphtha, B.P. 50-60°, contains chiefly pentane and hexane.
4. Benzoline or Benzine, B.P. 70-90°, contains chiefly hexane and heptane.
5. Ligroin or " petrol," B.P. 90-120°⎫ contain chiefly heptane and octane.
6. Petroleum Benzine, B.P. 120-150°⎭
7. Paraffin oil or Kerosene, B.P. 150-300°, contains chiefly octane to hexadecane.
8. Vaseline, B.P. above 300°, contains chiefly heptadecane to heneicosane ($C_{21}H_{44}$).

The fractions may be purified by shaking with concentrated sulphuric acid and caustic soda to remove unsaturated hydrocarbons.

The portions of higher boiling-point are decomposed by overheating, or by distilling under pressure (cracking process), and yield fractions of lower boiling-point.

The other natural mineral oils, found in Russia, Roumania, etc., are

also distilled fractionally. They contain generally less of the lower boiling fractions and a greater quantity of naphthene hydrocarbons.

Liquid hydrocarbons are also prepared by the distillation of bituminous shale.

The higher members, which are solid, remain as distillation residues, and are also found in nature, e.g. ozokerit.

The distillation residues are converted into oil and paraffin wax by freezing and pressing ; the liquid portion forms lubricating oil and the solid portion paraffin wax. The residues and fractions may be purified by treatment with sulphuric acid and caustic soda.

Examination of a Commercial Specimen of Hydrocarbons by Fractional Distillation.

On distilling 50-100 c.c. of ligroin, or kerosene, from a small distilling flask attached to a condenser and observing the temperature indicated by the thermometer, it will be seen that the temperature never remains constant for any length of time. The substance is a mixture. Several fractions which boil within 10° or 20° ranges of temperature can be collected in separate receivers. By redistilling these fractions and using a fractionating column (see p. 8) a pure product with a constant boiling-point can eventually be obtained.

Properties.

The saturated hydrocarbons have a peculiar odour. They are insoluble in water, but are soluble in alcohol, ether, and other organic liquids.

Inflammability.

Marsh gas and the other gases burn with a non-luminous flame and form explosive mixtures when mixed with a certain proportion of oxygen, or air.

The lower members amongst the liquids are also inflammable and burn with a more or less luminous flame. E.g. if about 3 c.c. of ligroin be placed in a watch glass and a lighted match applied it will burn.

The higher liquid members do not burn until they have been warmed. E.g. on applying a lighted match to about 3 c.c. of kerosene contained in a watch glass, the flame is extinguished, but if the kerosene be warmed on the water-bath to about 40° and a lighted match again applied, the vapours of the kerosene will be ignited.

In a lamp the kerosene rises to the surface of the wick by capillarity, and on applying a light the oil becomes hot and turns into inflammable vapour.

Inertness towards Chemical Reagents.

The saturated hydrocarbons are very inert substances; they are not acted upon by concentrated acids, or alkalies, except under special conditions, and on account of their stability they are known as the paraffins from *parum affinis*, little affinity.

E.g. on shaking about 1 c.c. of ligroin, or kerosene, with

(*a*) concentrated sulphuric acid

(*b*) concentrated nitric acid,

(*c*) potassium permanganate solution,

(*d*) bromine dissolved in chloroform,

there is no reaction unless the commercial mixture of hydrocarbons contain hydrocarbons belonging to the unsaturated series.

They are acted upon by the halogens forming substitution products (p. 49).

Synthetical Preparation.

1. The lower members of the series can be prepared by the action of water on certain metallic carbides, e.g. :—

Marsh gas is evolved if about 2 gm. of aluminium carbide in a test tube be covered with water. The gas may be collected in an inverted test tube and shown to be inflammable.

2. Saturated hydrocarbons can be prepared by the dry distillation of the dry sodium salt (1 part) of a fatty acid with soda lime (3 parts).

E.g. Methane is given off when fused sodium acetate and soda lime in the above proportions are heated together in a test tube. The evolved gas may be ignited at the mouth of the tube :—

$$CH_3COONa + NaOH = CH_4 + Na_2CO_3.$$

3. Saturated hydrocarbons are prepared from the corresponding halogen derivative by reduction with hydriodic acid, zinc-copper couple, zinc and hydrochloric acid, etc. :—

$$C_2H_5I + HI = C_2H_6 + I_2$$
$$C_2H_5I + 2H = C_2H_6 + HI.$$

4. The higher members of the series are prepared from the lower members by treating the dry halogen derivative (alkyl halide) with zinc or with sodium :—

$$CH_3I + 2Na + CH_3I = CH_3 . CH_3 + 2NaI$$
$$2CH_3I + Zn = CH_3 . CH_3 + ZnI_2.$$
$$CH_3I + Zn + CH_3 . CH_2I = CH_3 . CH_2 . CH_3 + ZnI_2$$
$$CH_3 . CH_2I + Zn + CH_3 . CH_2I = CH_3 . CH_2 . CH_2 . CH_3 + ZnI_2.$$
$$CH_3 . CH_2 . CH_2I + Zn + CH_3I = CH_3 . CH_2 . CH_2 . CH_3 + ZnI_2.$$
$$CH_3 . CHI . CH_3 + Zn + CH_3I = CH_3 . CH{\Large<}^{CH_3}_{CH_3} + ZnI_2.$$
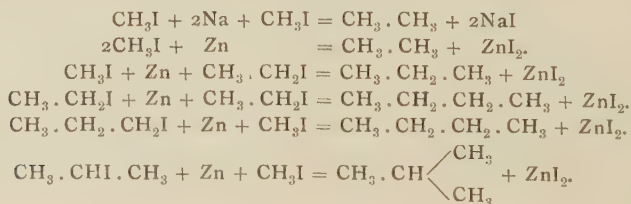
B. UNSATURATED.

The other series of hydrocarbons, the empirical formulæ of which are given on p. 39, contain less hydrogen in the molecule and are represented by the general formulæ C_nH_{2n} (olefines) and C_nH_{2n-2} (acetylenes). The two compounds ethylene or ethene, C_2H_4, and acetylene or ethine, C_2H_2, are the first and typical examples. In these compounds the four valencies of the carbon atoms are not completely satisfied by hydrogen atoms and they are therefore termed the unsaturated hydrocarbons. The unsaturated hydrocarbons are given the suffix *ene* and *ine* respectively. They are represented by graphic formulæ, in which the carbon atoms are joined by double or triple bonds :—

$$
\begin{array}{cccc}
CH_2 & CH & CH_3\text{—}CH\text{=}CH_2 & CH_3\text{—}C \equiv CH \\
\| & \| \| & \text{Propylene.} & \text{Propine.} \\
CH_2 & CH & & \\
\text{Ethylene.} & \text{Acetylene.} & &
\end{array}
$$
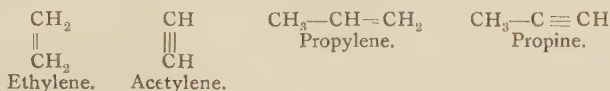
It should be noted that the double and the triple bonds do not indicate greater, but on the contrary lesser stability.

The carbon atoms in the olefine series are joined by their sides, in the acetylene series by their bases :—
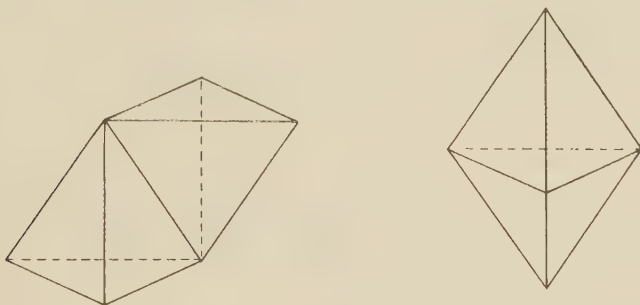
Isomers exist amongst the higher members in which the double bond occupies different positions in the chain :—

$$
\begin{array}{cc}
CH_3\text{—}CH_2\text{—}CH\text{=}CH_2 & CH_3\text{—}CH_2\text{—}C \equiv CH, \\
\alpha\text{-Butylene, or} & \alpha\text{-Butine, or} \\
\text{ethyl-ethylene} & \text{ethyl-acetylene} \\
CH_3\text{—}CH\text{=}CH\text{—}CH_3 & CH_3\text{—}C \equiv C\text{—}CH_3 \\
\beta\text{-butylene, or} & \beta\text{-butine, or} \\
\text{dimethyl-ethylene} & \text{dimethyl-acetylene,}
\end{array}
$$

$$
\begin{array}{c}
CH_3 \\
 \diagdown \\
 \diagup C\text{=}CH_2 \\
CH_3 \\
\text{Isobutylene.}
\end{array}
$$
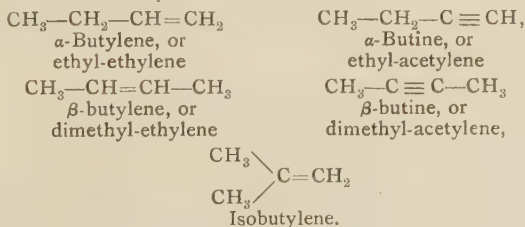
Further, compounds are known which contain two or more double bonds in their molecule, e.g.

$$
\begin{array}{c}
CH_2 \\
 \diagdown \\
 C\text{—}CH\text{=}CH_2 \\
CH_3 \diagup \\
\text{Isoprene.}
\end{array}
$$
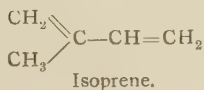
(a) OLEFINES.

Preparation.

1. The olefines are most usually prepared by abstracting the elements of water from alcohols (p. 57) by means of dehydrating agents —zinc chloride, sulphuric acid, phosphoric acid :—

$$C_2H_5OH = CH_2{=}CH_2 + H_2O.$$
$$C_3H_7OH = CH_3{-}CH{=}CH_2 + H_2O.$$
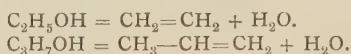
The preparation of ethylene by this method is described on p. 51.

2. Olefines are prepared by the action of alcoholic potash upon the alkyl halide (p. 49).

$$C_2H_5I + KOH = CH_2{=}CH_2 + H_2O + KI.$$
$$C_3H_7I + KOH = CH_3{-}CH{=}CH_2 + H_2O + KI.$$
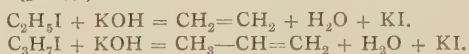
Ethylene may be prepared as follows :—

50 c.c. of a 20 per cent. solution of caustic potash in alcohol is placed in a 250 c.c. distilling flask in the neck of which a tap funnel is fastened with a cork. The distilling flask is fixed on a stand at an angle so that its neck may be attached to an inverted condenser (or its neck bent at an angle). A glass tube suitably bent leads from the condenser to a water trough. The potash solution is warmed and about 15 c.c. of ethyl iodide are slowly dropped in. Ethylene is evolved and potassium iodide is precipitated. When all the air has been displaced from the apparatus the gas may be collected in a gas cylinder over water.

Note.—Ether is formed in the reaction according to the equation :—

$$KOC_2H_5 + C_2H_5I = KI + C_2H_5 . O . C_2H_5.$$
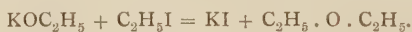
Properties.

The lower members with 2, 3, and 4 atoms of carbon are gases. The higher members are liquids and solids. They are lighter than water in which they are only slightly soluble. They are soluble in alcohol, ether, and other organic liquids. They are inflammable and burn with a luminous smoky flame.

Addition Reactions.

(1) **Hydrogen.** When mixed with hydrogen and passed through a hot tube over platinum black, or finely divided nickel, they are converted into saturated hydrocarbons :—

$$CH_2{=}CH_2 + H_2 = CH_3{-}CH_3.$$

The catalyst can be suspended in an inert solvent and a mixture of ethylene and hydrogen bubbled through the liquid.

(2) **Halogens.** The olefines combine with the halogens, chlorine and bromine, but less readily with iodine, to form halogen compounds containing two atoms of halogen (see p. 51) :—

$$CH_2{=}CH_2 + Br_2 = CH_2Br . CH_2Br.$$

(3) **Halogen Acids.** The following reaction occurs :—

$$CH_2{=}CH_2 + HI = CH_3 . CH_2I.$$

(4) **Sulphuric Acid.** The alkyl hydrogen sulphate (p. 67) is formed by addition :—

$$CH_2{=}CH_2 + H_2SO_4 = CH_3 . CH_2 . HSO_4.$$
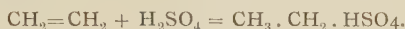
Note.—This reaction serves for the separation of saturated and unsaturated hydrocarbons.

(5) **Hypochlorous Acid.** Chlorhydrins are formed :—

$$CH_2{=}CH_2 + HOCl = CH_2OH . CH_2Cl$$
$$\text{Ethylene chlorhydrin.}$$
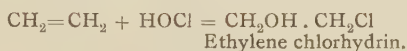
(6) **Potassium Permanganate.** The olefines are oxidised by dilute permanganate :—

$$CH_2{-}CH_2 + H_2O + O = CH_2OH . CH_2OH$$
$$\text{Ethylene glycol.}$$
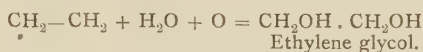
This reaction may be used for detecting unsaturated compounds in a mixture of hydrocarbons.

(b) ACETYLENES.

Acetylene is the only member of this group of any importance.

Preparation.

Acetylene was first prepared by sparking carbon electrodes in hydrogen. It is formed by the incomplete combustion of other hydrocarbons and is produced when a Bunsen burner strikes back.

Acetylene is most usually prepared by the action of water upon calcium carbide :—

$$CaC_2 + H_2O = C_2H_2 + CaO.$$

The hydrocarbons of this group are prepared by the action of alcoholic potash upon halogen compounds in the same way as ethylene :—

$$\begin{matrix} CH_2Br \\ | \\ CH_2Br \end{matrix} + KOH = \begin{matrix} CHBr \\ \| \\ CH_2 \end{matrix} + KBr + H_2O$$
$$\text{Vinyl bromide.}$$
$$\begin{matrix} CHBr \\ \| \\ CH_2 \end{matrix} + KOH = \begin{matrix} CH \\ \||| \\ CH \end{matrix} + KBr + H_2O.$$
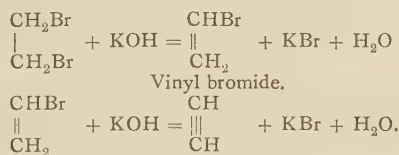
Properties.

The lower members are gases, the higher members are liquids. Acetylene is soluble in water (1 : 1) and other organic liquids. Acetone dissolves thirty-one times its own volume of the gas at N.T.P. Acetylene burns with a smoky, intensely hot flame which is very luminous; it is consequently employed for illuminating purposes, the burners, generally of clay, being designed so that complete combustion is effected.

Acetylene and the other members of the series form characteristic compounds with copper, silver, and other heavy metals.

Cuprous acetylide, C_2Cu_2, and silver acetylide, C_2Ag_2, are precipitated

as amorphous compounds when acetylene is passed through ammoniacal solutions of cuprous chloride, or silver nitrate. In the dry state these compounds are very explosive; they are decomposed on treatment with hydrochloric acid, or potassium cyanide, yielding acetylene. Acetylene may be separated from other hydrocarbons by this property.

Addition Reactions.

Acetylene and its homologues behave like the olefines, but react with two molecules :—

(1) **Hydrogen.**
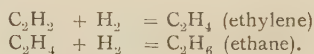
$$C_2H_2 + H_2 = C_2H_4 \text{ (ethylene)}$$
$$C_2H_4 + H_2 = C_2H_6 \text{ (ethane).}$$

(2) **Halogen Acid.**

$$C_2H_2 + HCl = \begin{matrix} CH_2 \\ \| \\ CHCl \end{matrix} \text{ (vinyl chloride)}$$

$$\begin{matrix} CH_2 \\ \| \\ CHCl \end{matrix} + HCl = \begin{matrix} CH_3 \\ | \\ CHCl_2 \end{matrix} \text{ (ethylidene chloride).}$$

(3) **Halogens.**

$$\begin{matrix} CH \\ \||| \\ CH \end{matrix} + Br_2 = \begin{matrix} CHBr \\ \| \\ CHBr \end{matrix} \text{ (acetylene dibromide)}$$

$$\begin{matrix} CHBr \\ \| \\ CHBr \end{matrix} + Br_2 = \begin{matrix} CHBr_2 \\ | \\ CHBr_2 \end{matrix} \text{ (ethane tetrabromide).}$$
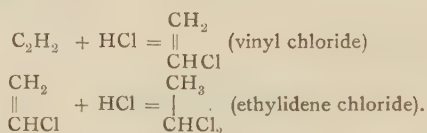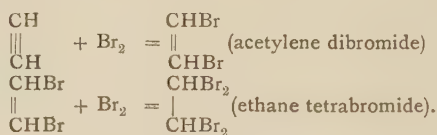
CHAPTER IV.

THE chief chemical property of the saturated hydrocarbons is that they are attacked by the halogens chlorine and bromine, yielding halogen substitution derivatives, one atom of hydrogen being progressively replaced by an atom of halogen; hydrogen chloride or bromide is formed at each stage of the reaction, thus,

$$CH_4 + Cl_2 = HCl + CH_3Cl, \text{ methyl chloride}$$
$$CH_3Cl + Cl_2 = HCl + CH_2Cl_2, \text{ methylene chloride}$$
$$CH_2Cl_2 + Cl_2 = HCl + CHCl_3, \text{ chloroform}$$
$$CHCl_3 + Cl_2 = HCl + CCl_4, \text{ carbon tetrachloride.}$$
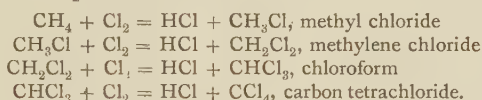
A mixture of the compounds results and the reaction is slow, so that, in practice, these compounds are not prepared from the hydrocarbon, but from other compounds.

The unsaturated hydrocarbons differ from the saturated hydrocarbons in their behaviour to the halogens. They react by addition, thus, e.g. ethylene combines with two atoms of bromine, forming the saturated compound, ethylene dibromide (p. 51):—

$$\begin{array}{cc} CH_2 & CH_2Br \\ \| & + Br_2 = | \\ CH_2 & CH_2Br. \end{array}$$
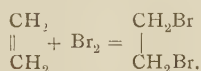
MONOHALOGEN DERIVATIVES. ALKYL HALIDES.

Preparation.

The alkyl halides are prepared from the corresponding alcohol by the action of the halogen acid, or by the action of the phosphorus halide:—

$$CH_3OH + HCl = CH_3Cl + H_2O$$
$$C_2H_5OH + HBr = C_2H_5Br + H_2O$$
$$3CH_3OH + PI_3 = CH_3I + H_3PO_3$$
$$CH_3OH + PCl_5 = CH_3Cl + POCl_3 + HCl.$$
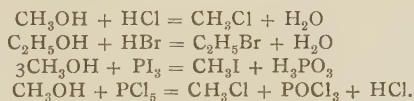
The reaction between the alcohol and hydrochloric acid is reversible

$$CH_3OH + HCl \rightleftarrows CH_3Cl + H_2O.$$

It is made to go to completion by removal of the water, usually by means of zinc chloride. Phosphorus and bromine, or iodine, are used to produce the phosphorus halide.

49

There are two monohalogen derivatives of propane,

$$CH_3 . CH_2 . CH_2I \qquad \text{and} \qquad CH_3 . CHI . CH_3$$
$$\text{propyl iodide} \qquad\qquad\qquad \text{isopropyl iodide.}$$
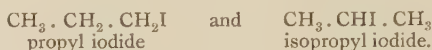
They are isomers differing in the position of the halogen atom in the molecule.

They can be prepared from the corresponding alcohol (p. 64).

Isopropyl iodide is commonly prepared from glycerol by the action of hydriodic acid, or phosphorus and iodine :—

$$CH_2OH . CHOH . CH_2OH + 5\,HI = 2I_2 + 3H_2O + CH_3 . CHI . CH_3.$$

Similar isomers occur in the case of the derivatives of butane and the higher hydrocarbons.

These compounds are also the esters of the corresponding alcohols with halogen acids (p. 67.).

Preparation of Methyl Chloride, or Ethyl Chloride.

Dry hydrogen chloride is passed into methyl alcohol, or ethyl alcohol, contained in a flask fitted with a reflux condenser and to which zinc chloride has been added. Methyl chloride is a gas and is evolved and may be collected over water. Ethyl chloride is a liquid with low boiling-point. The evolved gas, after passage through soda lime, is condensed in a **U** tube placed in a freezing mixture.

Preparation of Methyl Iodide, or Ethyl Iodide.

18 gm. of methyl alcohol and 5 gm. of red phosphorus are placed in a small flask (250 c.c.) and a reflux condenser is attached to it. 50 gm. of iodine are slowly added by detaching the flask from the condenser and rapidly refixing. Heat is evolved in the reaction and loss of alcohol and iodide is prevented by the condenser. The apparatus and mixture is allowed to stand for 12-24 hours so that the reaction completes itself. The contents of the flask are distilled from a water-bath. The distillate is shaken in a separating funnel with dilute caustic soda to remove iodine and hydriodic acid, and if sufficient has been used the lower layer of methyl iodide will be colourless. The lower layer of methyl iodide is separated, allowed to stand with a little calcium chloride till it is clear, and distilled from a water-bath (b.p. 44°).

About 45 gm. or 75 per cent. of the theoretical yield should be obtained.

Preparation of Ethyl Bromide.

A distilling flask of about 1 litre capacity is closed by a cork and its neck attached to a condenser. To the end of the condenser is attached an adapter (a bent tube wide at one end and narrow at the other, p. 11) which dips under water contained in a conical flask of about 250 c.c. capacity cooled by standing in ice. 54 c.c. (100 gm.) of sulphuric acid are mixed in the flask with 75 c.c. (60 gm.) of absolute alcohol and cooled to the temperature of the air. 100 gm. of coarsely powdered potassium bromide are added to the contents of the flask and the mixture is heated on a sand bath or carefully on a gauze. The contents boil and froth up and heavy oily drops of ethyl bromide collect under the water in the receiver. If the frothing is too great the flask is removed from the source of heat for a minute.

The heating is continued so long as oily drops distil over. The contents of the receiver are placed in a separating funnel and the lower layer collected. It is purified by returning to the separating funnel and shaking with a dilute solution of sodium carbonate. The ethyl bromide is then shaken with water to remove alkali, and it is placed in a clean dry distilling flask and left in contact with calcium chloride till it is clear. The flask is furnished with a thermometer, attached to a condenser and the ethyl bromide (b.p. 35-40°) distilled over from a water-bath. About 75-80 gm. should be obtained.

Ethyl bromide may also be prepared by means of phosphorus and bromine, in an analogous way to methyl iodide.
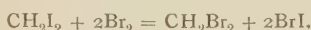
DIHALOGEN DERIVATIVES.

The dihalogen derivatives of methane are of little importance.

Methylene chloride, CH_2Cl_2, is generally prepared by reducing chloroform in alcoholic solution with zinc and hydrochloric acid.

Methylene iodide, CH_2I_2, is prepared by reducing iodoform with hydriodic acid.

Methylene bromide, CH_2Br_2, is prepared by treating methylene iodide with bromine :—

$$CH_2I_2 + 2Br_2 = CH_2Br_2 + 2BrI.$$

There are numerous isomers of the dihalogen derivatives of the higher hydrocarbons. The two isomers,

(a) CH_2Br and   (b) CH_3
     | |
    CH_2Br $CHBr_2$
    Ethylene   Ethylidene
    Bromide   Bromide
       or
    dibromide
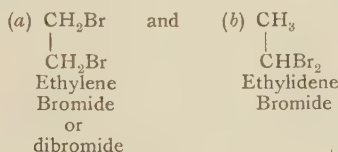
are derived from ethane and are prepared

(a) by addition of halogen to unsaturated hydrocarbons ;

(b) by the action of phosphorus pentachloride upon acetaldehyde :—

$$CH_3.CHO + PCl_5 = CH_3.CHCl_2 + POCl_3.$$

Preparation of Ethylene Dibromide.

Ethylene is prepared by dropping a mixture of 30 c.c. of absolute alcohol and 80 c.c. sulphuric acid from a tap funnel upon a mixture of 124 c.c. of alcohol and 108 c.c. of concentrated sulphuric acid contained in a 2 litre flask and mixed with sand to prevent frothing, the mixture being gently heated until a steady stream of gas is evolved. The evolved gas is passed through two wash bottles[1] with safety tubes containing caustic soda solution to remove sulphur dioxide into two ordinary wash bottles containing 50 c.c. bromine and 1 c.c. of water and 15 c.c. of bromine and 1 c.c. of water respectively. These two bottles are placed in a basin of water to which ice may be added to prevent the contents becoming warm during the reaction. The outlet tube is connected to a tower containing soda lime so that bromine vapour does not escape into the room. The bromine in the bottles is gradually decolorised and changes into ethylene bromide which may have a straw-yellow colour.

[1] It may be necessary to change these bottles for fresh ones during the preparation.

The heavy liquid product is shaken in a separating funnel with dilute caustic soda solution and then with water. It is dried with calcium chloride and purified by distillation (b.p. 130-132°). About 60 gm. should be obtained.

Properties of the Monohalogen and Dihalogen Derivatives.

Methyl chloride is a gas, easily condensed to a liquid. Liquid methyl chloride is sometimes used in minor surgical operations as a local anæsthetic. The part is frozen by a jet of methyl chloride which is allowed to impinge upon it, and insensibility so produced.

Methyl bromide and ethyl chloride are low boiling liquids.

The other monohalogen derivatives are also liquids. They are heavier than water in which they are insoluble, or only slightly soluble. They have a peculiar smell. The lower members burn with a green flame; the higher members do not burn readily. Their physical properties are in general like those of chloroform (p. 53).

Chemically, the monohalogen derivatives are very reactive substances, and readily exchange the atom of halogen with other atoms or groups, thus :—

1. $CH_3I + 2H = CH_4 + HI$ (p. 44).
2. $CH_3I + Zn + CH_3I = C_2H_6 + ZnI_2$ (p. 44).
3. $CH_3I + 2Zn + CH_3I = CH_3 . Zn . CH_3 + ZnI_2$.
4. $CH_3I + KOH = CH_3OH + KI$ (p. 57).
        aqueous
5. $C_2H_5I + KOH = C_2H_4 + KI + H_2O$ (p. 46).
        alcoholic
6. $C_2H_5I + NH_3 = C_2H_5NH_2 + HI$ (p. 108).
7. $C_2H_5I + KCN = C_2H_5CN + KI$ (p. 119).
8. $C_2H_5I + KNO_2 = C_2H_5NO_2 + KI$.
9. $C_2H_5I + KHS = C_2H_5HS + KI$ (p. 75).

In these reactions the organic part of the molecule remains unchanged, and resembles the metal in inorganic reactions. Hence it was called an organic or compound radicle. It consists of the saturated hydrocarbon with one hydrogen atom less. As a general name, the term alkyl has been given to these compound radicles.

On account of their reactiveness the alkyl halides are largely used for introducing *alkyl* radicles into other compounds.

The dihalogen derivatives are very similar to the monohalogen derivatives in both their physical and chemical properties. Both the halogen atoms can be replaced by OH, NH_2, etc.

TRIHALOGEN DERIVATIVES.

The chief trihalogen derivatives are chloroform and iodoform.
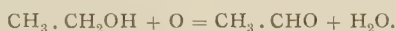
CHLOROFORM.

Preparation.

100 gm. of fresh bleaching powder are rubbed up in a mortar with 200 c.c. of water so as to form a paste, the paste is rinsed into a large

flask of about 1000 c.c. capacity with another 200 c.c. of water ; 25 c.c. of acetone, or alcohol, are added and the mixture shaken up thoroughly. The flask is connected by means of a bent tube to a condenser and receiver and gently heated through a wire gauze. As soon as the reaction commences, which is shown by the frothing, the flame is removed. When the frothing has subsided and the reaction has moderated, the contents of the flask are boiled until no more chloroform distils over with the water. The chloroform consists of heavy oily drops which sink in water, and it forms the lower layer of the distillate.

The distillate is transferred to a separating funnel and shaken with a little dilute caustic soda solution ; the lower layer of chloroform is drawn off into a clean, dry flask and dried by adding anhydrous calcium chloride, either by shaking for 5-10 minutes, or allowing to stand from 12-24 hours, until it is clear. The chloroform is filtered into a clean, dry distilling flask and distilled.

The mechanism of the reaction by which the chloroform is formed is probably :—

1. The oxidation of the alcohol to aldehyde (p. 77),
$$CH_3 . CH_2OH + O = CH_3 . CHO + H_2O.$$

2. The chlorination of the aldehyde to chloral,
$$CH_3 . CHO + 3Cl_2 = CCl_3 . CHO + 3HCl.$$
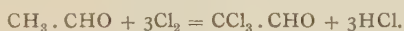
3. The decomposition of the chloral to chloroform and calcium formate by the calcium hydroxide (p. 85).
$$2CCl_3 . CHO + Ca(OH)_2 = 2CHCl_3 + (HCOO)_2Ca.$$
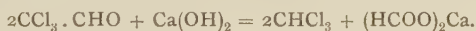
Purification of Commercial Chloroform.

Chloroform prepared from alcohol, methylated spirit (methylated chloroform), or acetone, may contain chlorine, hypochlorous acid or hydrochloric acid, aldehyde, etc.

The specimen is shaken several times with water, the chloroform is separated, dried with (1) calcium chloride, (2) phosphorus pentoxide and distilled.

The last traces of alcohol may also be removed by adding slices of metallic sodium, allowing to stand for 12-24 hours and then distilling.

* *Properties.*

Chloroform is a volatile colourless liquid with a distinct and sharp odour and sweetish taste. It boils at 61° and has a sp. gr. of 1·483-1·487.

* Its vapour does not burn, but when mixed with alcohol the combined vapours burn with a smoky flame edged with green.

It is soluble in about 200 volumes of cold water ('44 gm. in 100 c.c.) to which it gives a sweet taste.

It mixes in all proportions with absolute alcohol, ether, benzene, petroleum ether. It is slightly soluble in dilute alcohol and readily dissolves fats, resins, india-rubber, camphor, iodine, bromine.

Many specimens of commercial chloroform undergo oxidation on keeping, especially in the light, and are liable to contain chlorine, phosgene, hypochlorous acid or hydrochloric acid. This decomposition is hindered by the addition of 1 per cent. of alcohol. The bottle should be kept in the dark. 1 c.c. of chloroform on evaporation should leave no residue and if allowed to evaporate on clean filter paper should leave no disagreeable odour.

* Chloroform is decomposed by boiling with aqueous alkali, more rapidly in alcoholic solution, into alkali formate and chloride :—

$$CHCl_3 + 4NaOH = HCOONa + 3NaCl + 2H_2O.$$

* A few drops of chloroform are heated with dilute caustic soda. The presence of chloride is tested for in a small portion of the solution, the remainder is neutralised exactly, if it be still alkaline, and heated with mercuric chloride solution. A deposit of mercurous chloride and mercury shows the presence of formate.

Tests for Impurities in Chloroform.

* A quantity of the specimen is shaken up with two volumes of water. The water is separated and silver nitrate is added. Pure chloroform gives no reaction, but a precipitate of silver chloride indicates the presence of chlorides. If, on heating, the precipitate blackens the presence of aldehyde, or formic acid, is indicated. The water should not react with blue litmus.

* Chloroform is not soluble in concentrated sulphuric acid. Any darkening which occurs on shaking them together is due to the presence of aldehyde, methyl alcohol, etc. The presence of alcohol in chloroform may be detected by shaking some of the specimen with five volumes of water, filtering through a wet paper, and testing for alcohol in the filtrate by the iodoform reaction (p. 63).

Tests for Chloroform.

* (1) A red or yellow precipitate of cuprous oxide is formed on adding some solution of chloroform in water to Fehling's solution (p. 83) and heating.

* (2) *Carbylamine Reaction.*—To the dilute solution of chloroform in water is added some alcoholic sodium hydroxide and a drop of

aniline and the mixture heated.   Phenyl isonitrile, or carbylamine, is formed, which has a disgusting smell :—

$$CHCl_3 + 3KOH + C_6H_5NH_2 = C_6H_5NC + 3KCl + 3H_2O.$$
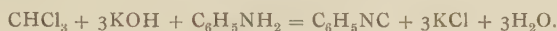
This reaction is extraordinarily sensitive and will detect one part of chloroform in 5000 parts of alcohol.   It is also given by bromoform, iodoform, chloral, trichloracetic acid and substances which yield chloroform when treated with alkali.

From liquids, such as blood, it is better to remove the chloroform as described under estimation and to test the liquid in the receiver.

Estimation of Chloroform.

Hydrochloric acid is formed when chloroform vapour mixed with hydrogen is passed through a red-hot tube.

Hydrogen is slowly passed into a flask containing the solution of chloroform and the flask is gently heated.   The mixed vapours are passed through a short, heated combustion tube containing platinum wire gauze or loose asbestos and into a receiver containing water.   The contents of the receiver are titrated with standard alkali, or precipitated with silver nitrate.   As acetylene and hydrogen cyanide may also be present, the contents of the receiver should be boiled before titrating, or precipitating.

This procedure may be used for detecting and estimating chloroform in blood and other liquids which do not contain other chlorinated compounds.

IODOFORM.

Preparation.

4 gm. of crystallised sodium carbonate are dissolved in 20 c.c. of water.   2 c.c. of absolute alcohol and 2 gm. of iodine are added, and the solution warmed to about 70° on the water-bath until it is decolorised.   Iodoform separates as a lemon-yellow powder.   It is filtered off, washed with cold water, and dried on an unglazed plate.

The melting-point of the preparation serves to prove its identity.

Properties.

Iodoform is a light yellow, shining crystalline solid with a persistent unpleasant odour.   It has a characteristic microscopic appearance—hexagonal plates, stars, or rosettes, and melts at 119°.   On gently heating it sublimes without change, but on heating strongly it is decomposed : violet vapours of iodine are formed and a deposit of carbon is left.

Iodoform is nearly insoluble in water (1 part in 10,000) and in dilute acids and alkalies.   It is slightly soluble in alcohol (1 part in 50) but more easily soluble in absolute alcohol (1 part in 23).   It is easily soluble in ether, chloroform, and carbon disulphide, but very slightly

soluble in glycerol, benzene, and petroleum ether.   In its chemical properties iodoform closely resembles chloroform.

Tests for Impurities in Iodoform.

1. No residue should remain when it is heated in the air.

2. It should be completely soluble in boiling alcohol, but insoluble in brine.

3. On shaking up with water and filtering, the filtrate should give no reaction with barium chloride or silver nitrate.

4. If picric acid be present as adulterant, it may be detected (*a*) By testing the aqueous extract with potassium cyanide when a reddish-brown coloration is produced. (*b*) By treating with caustic soda solution and shaking this solution with chloroform.   Picric acid remains in the aqueous solution. (*c*) By extracting the acid with dilute sodium carbonate solution, neutralising exactly with acetic acid and adding potassium nitrate ; potassium picrate is precipitated.

CHAPTER V.

ALCOHOLS may be regarded as hydrocarbons in which a hydrogen atom (or more in the case of the higher members, e.g. glycerol) has been substituted by a hydroxyl, or OH, group. This relationship is shown : By the action of water, or aqueous alkalies, upon the halogen mono-substituted hydrocarbons, or alkyl halides :—

$$CH_3 . Cl + HOH = HCl + CH_3 . OH.$$

We can thus pass from the hydrocarbon to the alcohol :—

$$CH_4 \rightarrow CH_3Cl \rightarrow CH_3OH$$
$$C_2H_6 \rightarrow C_2H_5Cl \rightarrow C_2H_5OH.$$
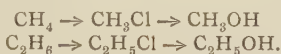
Conversely, by the action of phosphorus pentachloride upon the alcohol, the alkyl halide is obtained :—

$$CH_3 . OH + PCl_5 = CH_3 . Cl + POCl_3 + HCl.$$

The hydrocarbon can be obtained from the alkyl halide by reduction (p. 44).

$$CH_3OH \rightarrow CH_3Cl \rightarrow CH_4$$
$$C_2H_5OH \rightarrow C_2H_5Cl \rightarrow C_2H_6.$$
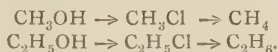
Alcohols are designated by the suffix -ol, e.g. methyl alcohol or methanol, ethyl alcohol or ethanol.

Two isomeric alcohols are derived from propane :—

$$CH_3 . CH_2 . CH_2OH \qquad CH_3 . CHOH . CH_3.$$
Propyl alcohol.   Isopropyl alcohol.
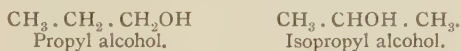
Four isomers are possible in the case of the butyl alcohols :—

CH$_2$OH	CH$_2$OH	CH$_3$	CH$_3$
CH$_2$	CH	CHOH	COH
CH$_2$	CH$_3$ CH$_3$	CH$_2$	CH$_3$ CH$_3$
CH$_3$		CH$_3$	
Normal primary butyl alcohol.	Primary isobutyl alcohol.	Normal secondary butyl alcohol.	Tertiary butyl alcohol.
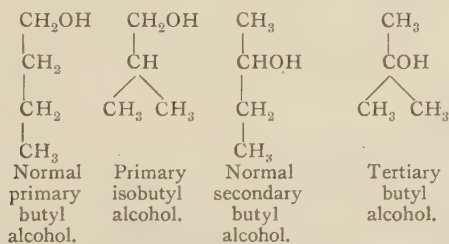
These isomeric alcohols differ (*a*) in the position of the OH group in the molecule, and (*b*) more particularly in the nature of the groups or atoms attached to the carbon atom upon which the OH group is situated.

Methyl, ethyl, propyl, and the first two butyl alcohols have the OH group attached to an end carbon atom of the series. The essential grouping is $-CH_2OH$, and it is known as a primary alcohol group.

Isopropyl alcohol and the third butyl alcohol have the OH group attached to a middle carbon atom. The essential grouping is $>CHOH$, a secondary alcohol group.

The fourth butyl alcohol has the OH group attached to a middle carbon atom. The essential grouping is $>C.OH$, a tertiary alcohol group.

Hydrogen may satisfy the free valency of $-CH_2OH$, giving methyl alcohol, but otherwise only alkyl groups (CH_3, C_2H_5, etc.) are attached to the free valencies.

The isomers differ in their boiling-points and specific gravities, thus :—

	B.P.	Sp. Gr.		B.P.	Sp. Gr.
Propyl	97°	·807	Isopropyl	82°	·792
Normal primary butyl	117°	·810	Primary isobutyl	107°	·806
Normal secondary butyl	100°	·808	Tertiary butyl	83°	·786

but their chief difference is in the behaviour on oxidation :—

Primary alcohols yield firstly aldehydes (p. 77) and then fatty acids containing the same number of carbon atoms in the molecule.

Secondary alcohols yield ketones (p. 87) and on further oxidation the molecule breaks up with the formation of acids containing fewer carbon atoms. Tertiary alcohols on oxidation decompose and yield ketones, or acids, with fewer carbon atoms in the molecule than the original alcohol.

The alcohols derived from the pentanes are known as amyl alcohols. Eight isomers are possible, all of which are known :—

	B.P.	Sp. Gr. at 20°.
1. Normal primary, $CH_3.CH_2.CH_2.CH_2.CH_2OH$	138°	·817
2. Isobutyl carbinol, $\begin{matrix} CH_3 \\ CH_3 \end{matrix}>CH.CH_2.CH_2OH$	130°	·810
3. Secondary butyl carbinol, $\begin{matrix} CH_3 \\ CH_3CH_2 \end{matrix}>CH.CH_2OH$	128°	·816
4. Tertiary butyl carbinol, (primary) $\begin{matrix} CH_3 \\ CH_3-C.CH_2OH \\ CH_3 \end{matrix}$	113°	. —
5. Methyl propyl carbinol, (secondary) $\begin{matrix} CH_3 \\ CH_3.CH_2.CH_2 \end{matrix}>CHOH$	119°	—
6. Methyl isopropyl carbinol, (secondary) $\begin{matrix} CH_3 \\ CH_3 \\ CH_3 \end{matrix}>CH>CHOH$	112°	·819

	B.P.	Sp. Gr. at 20°.

7. Diethyl carbinol,
   (secondary)

$$\begin{matrix} CH_3 . CH_2 \\ \end{matrix}\Big\rangle CHOH \qquad 117 \qquad —$$
$$CH_3 . CH_2$$

8. Dimethyl ethyl carbinol,
   (tertiary)

$$\begin{matrix} CH_3 \\ CH_3—C . OH \\ CH_3 . CH_2 \end{matrix} \qquad 102 \qquad —$$

Secondary butyl carbinol contains an asymmetric carbon atom (see under stereoisomerism) and consequently exists in a dextro- and a laevo-form.

Most of the alcohols are natural substances and serve as the starting-point for the preparation of other compounds.

METHYL ALCOHOL, $CH_3 . OH$.

Commercial Methyl Alcohol.

Preparation.

Methyl alcohol, together with acetone, acetic acid, methyl acetate and other substances is formed in the dry distillation of wood. The acid aqueous distillate is known as pyroligneous acid ; on standing wood tar separates out. The acid liquid contains 1-2 per cent. of methyl alcohol, ·1-5 per cent. of acetone and about 10 per cent. of acetic acid. It is distilled until the distillate has a specific gravity of ·9-1. The crude wood spirit so obtained is a greenish-yellow liquid with disagreeable odour. It is mixed with about 2 per cent. of lime and again distilled. This retains the acetic acid as calcium acetate, the neutral substances— methyl alcohol, acetone, acetaldehyde, methyl acetate passing over. This distillate is wood spirit and contains about 93 per cent. of methyl alcohol. It is diluted with water to precipitate oily impurities and is again treated with lime and distilled. Basic impurities are removed by distilling it with ·1-·2 per cent. of sulphuric acid and the methyl alcohol boiling at 64-66° is collected.

Methyl alcohol is also prepared by dry distillation from vinasses— the mass remaining after fermentation of the residues from the preparation of beet sugar.

Pure Methyl Alcohol.

Commercial methyl alcohol contains acetone. By dissolving anhydrous oxalic acid (prepared by heating oxalic acid at 100°) in the boiling spirit, methyl oxalate is formed ; it separates in crystals on cooling. The crystals are filtered off, freed from acetone, and then decomposed into oxalic acid and methyl alcohol by boiling with water or ammonia. Methyl alcohol is obtained on distillation and is dehydrated by distilling over quicklime (see under ethyl alcohol).

Pure methyl alcohol may also be obtained by boiling commercial methyl alcohol with anhydrous calcium chloride. Calcium chloride crystallises out in combination with methyl alcohol as $CaCl_2 + 4CH_3OH$ from the saturated solution on cooling. The crystals are drained from the mother liquor and are decomposed by heating ; methyl alcohol is evolved and is collected.

The acetone may also be removed by passing chlorine into it forming trichloracetone. The methyl alcohol is separated by fractional distillation.

Properties.

Methyl alcohol is a colourless liquid which boils at 66° and has a sp. gr. of ·797 at 15°. It closely resembles ethyl alcohol in its properties, but it does not give the iodoform reaction.

ETHYL ALCOHOL.

Preparation.

Ethyl alcohol is obtained by the fermentation of sugar by yeast and occurs in all fermented liquids such as wine and beer. It is made chiefly from potatoes and cereals, the starch being first converted into the sugar, glucose, which is fermented by the yeast and changed into alcohol and carbon dioxide.

*   About 50 gm. of sugar are dissolved in 500 c.c. of water in a 2-litre flask : 1-2 gm. of nutrient salts and about 15 gm. of baker's yeast are added. The flask is fitted with a bent tube leading to a beaker containing lime water. The apparatus is kept in a warm room for two to three days. The contents of the flask will show effervescence, and the production of carbon dioxide is shown by a deposit of calcium carbonate in the lime water. When effervescence and production of CO_2 ceases, fermentation is complete. The contents of the flask are poured into a distilling apparatus and distilled. The distillate is tested for alcohol (p. 63).

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$

1. Rectified Spirit.

The alcohol produced by fermentation is separated from the fermented liquor by distillation. The distillate is then fractionally redistilled, or rectified, so as to separate as much water as possible and the greater part of the higher alcohols. The product is rectified spirit. It contains about 84 per cent. by weight of ethyl alcohol and has a sp. gr. of 0·838 at 15°.

2. Methylated Spirit.

The rectified spirit is denatured and rendered unfit for drinking purposes by the addition to it of one-ninth of its volume of wood spirit and three-eighths of 1 per cent. of mineral naphtha, or paraffin oil.

Since 1905 methylated spirit has been obtainable in approved scientific institutions free of duty and free from mineral naphtha.

3. Absolute Alcohol.

Rectified spirit is filtered through charcoal and fractionally distilled, the first portions which contain aldehyde and the last portions which contain fusel oil being rejected. The middle fraction is distilled over quicklime and commercial absolute alcohol is obtained. This contains about ·5 per cent. of water. Pure alcohol is prepared from this by adding the requisite quantity of metallic sodium, or calcium, and again distilling.

4. Absolute Alcohol from Methylated Spirit.

Methylated spirit (1 litre) is boiled upon a water-bath under a reflux or inverted condenser (p. 13) with about 30 gm. of caustic soda for one hour in a 2-litre flask. Acetone, aldehyde and other impurities are destroyed and the spirit turns brown. The contents of the flask are distilled and the distillate collected in another flask of the same capacity containing about 100 gm. of quicklime. The flask is connected with a reflux condenser and either allowed to stand for twenty-four hours or heated for one hour on a water-bath. The liquid is distilled again without pouring off from the flask. The yield of absolute alcohol is about 80 per cent., and it contains 2-3 per cent. of water. By treating it again with half the previous quantity of quicklime the amount of water may be reduced to less than 1 per cent. The boiling° point (76-78°) may be determined by distilling 50 c.c. in a small apparatus.

Properties.

(1) Ethyl alcohol is a colourless, pleasant-smelling liquid with a hot taste. It boils at 78° and has a sp. gr. of ·79384 at 15·5- or 60° F.

(2) It mixes with water in all proportions. Absolute alcohol is very hygroscopic and readily absorbs water on exposure to the air.

On mixing alcohol with water, there is an evolution of heat and a contraction in bulk.

The addition of water to methylated spirit produces a cloudiness due to the precipitation of the mineral naphtha.

(3) Alcohol burns with a faint blue non-luminous flame even when mixed with considerable amounts of water.

＊   On mixing 10 c.c. of alcohol with 10 c.c. of water in a measuring cylinder the evolution of heat will be noticed, and when the mixture is cold the, diminution in volume can be measured. By placing the mixture in a small basin and applying a light it will be seen whether it is, or is not, inflammable.

Detection of Water in Absolute Alcohol.

＊   (a) If the alcohol contains a considerable quantity of water its presence will be shown by adding some anhydrous copper sulphate which turns blue.[1]

＊   (b) 0·5 per cent. of water may be detected by adding a crystal of potassium permanganate ; the liquid will assume a pink colour.

(c) Traces of water in absolute alcohol according to Yvon may be detected by means of calcium carbide. If water be present, bubbles of acetylene are given off and the liquid becomes milky, due to the formation of calcium hydroxide.

Reactions.

1. *Action of Sodium.*

＊   On adding about 1 gm. of sodium to 20 c.c. of absolute alcohol in a small flask there is an evolution of hydrogen just as with water, but the reaction is by no means so violent.   The gas which is evolved may be collected in an inverted test tube and shown to be hydrogen by burning.

When the sodium has dissolved the solution is evaporated to dryness on the water-bath.   A white solid—sodium ethoxide—remains, which is very hygroscopic and is decomposed by water, yielding alcohol which can be recognised by its smell and by the iodoform test :—

$$C_2H_5OH + Na = C_2H_5ONa + H$$
$$C_2H_5ONa + H_2O = C_2H_5OH + NaOH.$$
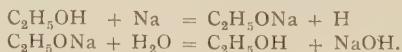
2. *Action of Phosphorus Pentachloride.*

＊   On adding a little phosphorus pentachloride to a small quantity of alcohol, a vigorous action cccurs and hydrochloric acid fumes are evolved.   Ethyl chloride and phosphorus oxychloride are the other products.   The smell of ethyl chloride will be noticed when the hydrochloric acid fumes cease to be given off :—

$$C_2H_5OH + PCl_5 = C_2H_5Cl + POCl_3 + HCl.$$

---

[1] Prepared by gently heating a crystal of copper sulphate in a crucible until it falls to powder.

Constitution of Alcohol.

These two reactions prove the constitution of alcohols. The action of sodium shows that only one of the hydrogen atoms in alcohol is replaceable by sodium. The action of PCl_5 shows the replacement of an hydroxyl, or OH, group.

If PCl_5 be allowed to act upon sodium ethoxide, ethyl chloride is again obtained :—

$$C_2H_5ONa + PCl_5 = C_2H_5Cl + POCL_3 + NaCl.$$

The hydrogen atom replaced by sodium is attached to oxygen.

Tests.

* (1) *Smell.*—Even in dilute solutions alcohol may be detected by its smell.

* (2) *Oxidation to Acetaldehyde.*—On warming a little dilute alcohol in a test tube with a few drops of potassium dichromate and some dilute sulphuric acid, the pungent characteristic odour of aldehyde will be observed and the solution turns green :—

$$C_2H_5OH + O = CH_3 . CHO + H_2O.$$

* (3) *Formation of Ethyl Acetate.*—The fruity odour of ethyl acetate is produced when some of the dilute solution is heated with concentrated sulphuric acid and a little anhydrous sodium acetate.

* (4) *Iodoform Reaction* (Lieben).—About an equal volume of iodine in potassium iodide is added to a very dilute solution of alcohol—1 or 2 drops in half a test tube full of water—and then, drop by drop, caustic soda till the mixture is decolorised. On gently warming the mixture, iodoform is formed and may be recognised by its characteristic smell. A yellow crystalline precipitate will separate if the solution of alcohol is not too weak.

Note.—This very sensitive reaction is not characteristic of alcohol as it may be given by aldehyde, acetone, acetic ester and other substances which contain the grouping CH_3—C joined to oxygen.

Alcohol gives the reaction on warming, acetone gives the reaction in the cold.

Estimation of Alcohol in Beer, Wines, Spirits.

The amount of alcohol in these liquids is ascertained by distilling off the alcohol and determining the specific gravity of the distillate.

(a) 100 c.c. beer are distilled and 80 c.c. distillate are collected.
(b) 100 c.c. wine + 80 c.c. water and a little tannin are distilled and nearly 100 c.c. distillate are collected.
(c) 50 c.c. spirit + 100 c.c. water, or 25 c.c. spirit + 150 c.c. water, are distilled and nearly 100 c.c. distillate are collected.

The distillate is made up to 100 c.c. with water, the liquids are mixed, and the sp. gr. at 15·5° or 60° F. is determined by weighing in a sp. gr. bottle. The amount is given by referring to an alcohol specific gravity table for the percentage by weight. The amount in the sample is ascertained from the formula :—

$$\frac{\text{sp. gr. of distillate} \times \text{amount of distillate in c.c.} \times \text{per cent. of alcohol from table}}{\text{sp. gr. of sample} \times \text{amount of sample taken}}$$

= percentage of abs. alc. by weight in the sample.

If the specific gravity of the sample be unknown, it may be calculated from

$$\frac{\text{wt. of distillate} \times \text{per cent. of alcohol from table}}{\text{wt. of sample taken}}$$

= percentage of abs. alc. by weight in the sample.

Normal Propyl Alcohol, $CH_3 . CH_2 . CH_2OH$.

Normal propyl alcohol is formed in the process of alcoholic fermentation and is contained in fusel oil, from which it is obtained by fractional distillation. It is present to the extent of about 3 per cent. in the fusel oil obtained from potato spirit.

It is a liquid resembling ethyl alcohol but with a less pleasant smell, and burns with a luminous flame.

Isopropyl Alcohol, $\genfrac{}{}{0pt}{}{CH_3}{CH_3}{\Large>}CHOH$.

Isopropyl alcohol is prepared either by the reduction of acetone (p. 87) with sodium amalgam, or from isopropyl iodide by boiling it with lead hydroxide and water. Isopropyl iodide is prepared by the action of phosphorus and iodine upon dilute glycerol.

It is a liquid resembling normal propyl alcohol.

BUTYL ALCOHOLS, C_4H_9OH.

The chief of these is primary isobutyl alcohol which is formed in alcoholic fermentation and is contained in fusel oil from which it is separated by fractional distillation.

Normal primary butyl alcohol has once been found in fusel oil. It is formed by the action of the *Schizomycetes* upon glycerol. *Bacillus butylicus* (contained in the excrement of cows) produces 6-8 per cent. from glycerol and 10 per cent. from mannitol. Normal secondary butyl alcohol and tertiary butyl alcohol are prepared by synthetical methods. The butyl alcohols are not miscible with water in all proportions ; normal primary butyl alcohol requires 12 parts of water to dissolve it.

AMYL ALCOHOLS, $C_5H_{11}OH$.

The two amyl alcohols, isobutyl carbinol and laevo-secondary butyl carbinol, together with primary isobutyl alcohol and propyl alcohol, are

the principal constituents of fusel oil—and they together constitute fermentation amyl alcohol.

Fermentation amyl alcohol is a strongly refractive liquid which boils at 130-131° and is very slightly soluble in water—3·3 volumes in 100 volumes of water at 22°. Its vapours, on being inhaled, produce a peculiar sensation in the head, causing headache, etc. The two alcohols cannot be separated by fractional distillation, but only by chemical means. The mixture, generally referred to as amyl alcohol, is frequently used as a solvent.

The fusel oil from potatoes or cereals contains chiefly isobutyl carbinol, secondary butyl carbinol being present only to 13-22 per cent. The fusel oil from beet molasses contains 48-58 per cent. of secondary butyl carbinol.

HIGHER ALCOHOLS.

A hexyl alcohol has been isolated from the fused oil obtained from grape skins. Two primary hexyl alcohols occur as esters : n-primary hexyl alcohol in the oil from the seeds of the parsnip, *Heracleum giganteum*, and 3-methyl-pentanol in Roman camomile oil.

Normal primary heptyl alcohol is prepared by the reduction of oenanthylic aldehyde which is obtained by distilling castor oil.

Normal primary octyl alcohol occurs in the oil from the fruits of the parsnips, *Heracleum sphondylium*, *Heracleum giganteum* and *Pastinaca sativa*.

Normal nonyl alcohol is prepared by reducing with sodium and alcohol methyl heptyl ketone which is contained up to 5 per cent. in oil of rue.

Normal secondary undecylic alcohol is prepared by reducing methyl nonyl ketone, which occurs in camomile in large quantities.

n-dodecyl alcohol occurs as ester in oil of *Cascara sagrada*.

Cetyl Alcohol.

Normal hexadecyl alcohol, or cetyl alcohol, $C_{16}H_{33}OH$, the most important of the higher alcohols, is easily prepared from spermaceti, in which it is present as ester, by hydrolysing the ester with alcoholic soda, diluting with water, filtering off and recrystallising the cetyl alcohol from alcohol. Cetyl alcohol has also been described as being present in the fat from an ovarian dermoid cyst. Cetyl alcohol is a white solid which melts at 50°.

Ceryl alcohol, $C_{27}H_{55}OH$, or more probably $C_{26}H_{53}OH$, is prepared from Chinese wax, and melts at 76-79°.

Myricyl alcohol, $C_{30}H_{61}OH$, is best prepared from carnauba wax. Beeswax contains this alcohol, or the alcohol $C_{31}H_{63}OH$.

Psylla-stearyl alcohol, $C_{33}H_{68}O$, has been obtained from the fat of the leaf louse (Psylla alni).

CHAPTER VI.

ALCOHOLS are like the bases NaOH, KOH in containing an OH group. The bases combine with acids to form salts. Alcohols combine with acids to form esters.

Alcohols differ from the inorganic bases (1) in having a neutral reaction, (2) in not being ionised in aqueous solution, (3) in that their reaction with acids is not instantaneous, but takes place slowly, and this reaction is reversible.

An enormous number of esters is possible since any alcohol can be combined with any acid, inorganic or organic.

Organic acids are characterised by the grouping—COOH and may be grouped into three classes : (a) those insoluble or very insoluble in water such as benzoic acid, $C_6H_5 . COOH$; (b) those soluble in water, but volatile with steam such as acetic acid, $CH_3 . COOH$; (c) those soluble in water, but not volatile with steam such as lactic acid, $CH_3 . CHOH . COOH$.
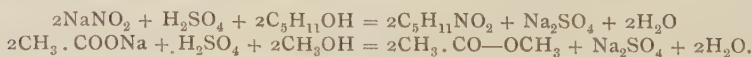
Preparation.

There are several methods of preparing esters :—

(1) By the action of the acid upon the alcohol in the presence of a dehydrating agent, or catalyst.

$$HCl(+ ZnCl_2) + C_2H_5OH = C_2H_5Cl + H_2O$$
$$CH_3 . COOH(+ H_2SO_4) + C_2H_5OH = CH_3 . CO—OC_2H_5 + H_2O.$$

(2) By the action of concentrated sulphuric acid upon the sodium salt of the acid and the alcohol.

$$2NaNO_2 + H_2SO_4 + 2C_5H_{11}OH = 2C_5H_{11}NO_2 + Na_2SO_4 + 2H_2O$$
$$2CH_3 . COONa + H_2SO_4 + 2CH_3OH = 2CH_3 . CO—OCH_3 + Na_2SO_4 + 2H_2O.$$

(3) By the action of the acid chloride, or anhydride, upon the alcohol.

$$COCl_2 + 2C_2H_5OH = CO(OC_2H_5)_2 + 2HCl$$
$$CH_3 . COCl + C_2H_5OH = CH_3 . CO—OC_2H_5 + HCl$$

$$\begin{matrix} CH_3 . CO \\ \diagdown \\ \diagup \\ CH_3 . CO \end{matrix} O + C_2H_5OH = CH_3 . CO—OC_2H_5 + CH_3 . COOH.$$
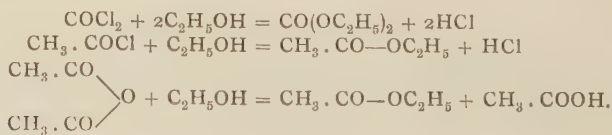
(4) By the action of the alkyl halide upon the silver salt.

$$C_2H_5Cl + CH_3.COOAg = AgCl + CH_3.CO—OC_2H_5.$$
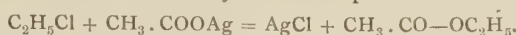
ESTERS OF INORGANIC ACIDS.

Halogen Acids.

These compounds are the same as the alkyl halides (p. 49) :—

$$C_2H_5OH + HCl = C_2H_5Cl + H_2O.$$
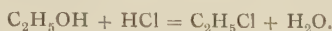
Amyl Nitrite.

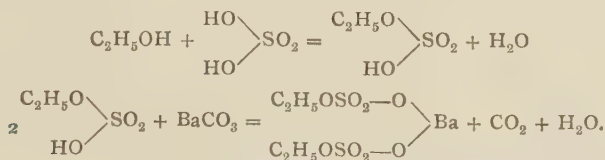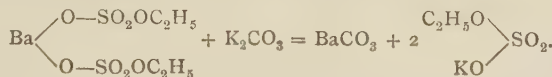The calculated quantity of concentrated sulphuric acid is allowed to drop slowly upon the calculated quantity of sodium nitrite mixed with the calculated quantity of amyl alcohol contained in a flask cooled by a freezing mixture. Amyl nitrite floats to the surface as an oil. It is separated, washed with water, dried with calcium chloride and distilled.

* Ethyl Sulphuric Acid.   Barium and Potassium Ethyl Sulphate.

10 c.c. of concentrated sulphuric acid are poured carefully into and mixed with 20 c.c. of ethyl alcohol ; the mixture becomes hot. It is heated on a water-bath under a reflux condenser for $\frac{1}{2}$-1 hour. On cooling it is poured into about 200 c.c. of cold water. The acid solution is neutralised to litmus by stirring it up with calcium, or barium, carbonate. Carbon dioxide is evolved and the excess of sulphuric acid is precipitated as insoluble sulphate ; this is filtered off after heating on a water-bath for $\frac{1}{2}$-1 hour. The solution contains calcium, or barium, ethyl sulphate (as shown below under hydrolysis).

$$C_2H_5OH + \begin{matrix} HO\\ \\ HO \end{matrix}\Big\rangle SO_2 = \begin{matrix} C_2H_5O\\ \\ HO \end{matrix}\Big\rangle SO_2 + H_2O$$

$$2\begin{matrix} C_2H_5O\\ \\ HO \end{matrix}\Big\rangle SO_2 + BaCO_3 = \begin{matrix} C_2H_5OSO_2—O\\ \\ C_2H_5OSO_2—O \end{matrix}\Big\rangle Ba + CO_2 + H_2O.$$

The clear filtrate is heated on the water-bath and treated with a strong solution of potassium carbonate (10 gm.), or potassium oxalate, until no further precipitate is formed :—

$$Ba\Big\langle \begin{matrix} O—SO_2OC_2H_5\\ \\ O—SO_2OC_2H_5 \end{matrix} + K_2CO_3 = BaCO_3 + 2\begin{matrix} C_2H_5O\\ \\ KO \end{matrix}\Big\rangle SO_2.$$

The barium carbonate is filtered off and the filtrate evaporated to a small volume (a drop withdrawn on a glass rod should crystallise on cooling). The crystals which form after standing for several hours are filtered off, washed with dilute alcohol and dried between sheets of filter paper. The mother liquor yields more crystals on further evaporation. The salt is dissolved in boiling alcohol under a reflux condenser, filtered, using a hot-water funnel, and allowed to crystallise out.

ESTERS OF ORGANIC ACIDS.

* **Ethyl Acetate.**

Molecular proportions of glacial acetic acid (50 c.c.) and absolute alcohol (50 c.c.) are mixed in a distilling flask, and 1 per cent. by volume of concentrated sulphuric acid (1 c.c.) is added. The distilling flask is connected to a condenser and receiver and the mixture is distilled. A yield of 86·5 per cent. of ester is obtained (Senderens' method). Ethyl sulphuric acid appears to be the catalyst :—

$$C_2H_5HSO_4 + C_2H_5OH = (C_2H_5)_2SO_4 + H_2O$$
$$(C_2H_5)_2SO_4 + CH_3COOH = C_2H_5HSO_4 + CH_3COOC_2H_5.$$
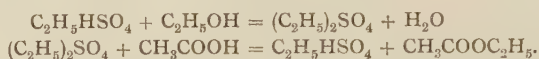
The distillate which contains water, alcohol and acetic acid is purified by shaking it in a separating funnel with strong sodium carbonate solution which is added in small quantities until the aqueous portion shows an alkaline reaction. The aqueous portion is withdrawn and the ester shaken with saturated salt, or strong calcium chloride, solution to remove alcohol ; the ester layer is separated and dried by contact with solid calcium chloride. It is then distilled from a dry flask, the portions passing over when the thermometer reads 74-78° being collected.

* **Ethyl Benzoate.**

20 gm. of benzoic acid are dissolved in 75 c.c. of absolute alcohol and 1 c.c. of concentrated sulphuric acid is added. The flask containing the mixture is connected to a reflux condenser and gently heated for 1-2 hours over a gauze, a piece of porcelain being added to prevent bumping. The esterification is complete when, on testing by pouring a few drops into water, only oil drops and no crystalline benzoic acid is seen. The whole is then poured into about 400 c.c. of water and the oil is allowed to settle. The water is decanted off and the remainder is shaken up with ether in a separating funnel. The aqueous layer is withdrawn, the ethereal layer shaken with sodium carbonate solution and then with water. It is dried with calcium chloride and the ether distilled off over a water-bath. The ester is distilled over a flame and the fraction boiling from 210-215° is collected. Ethyl benzoate boils at 213°.

Properties.

Esters are usually liquids having a sweet and fragrant odour ; a few are solid. Many esters occur naturally and are the sweet-smelling constituents of plants. They are manufactured as substitutes for the natural essence.

Neutral esters of monobasic, dibasic, etc., acids are insoluble in water, or only slightly soluble, e.g. ethyl formate and acetate.

Acid esters of dibasic, etc., acids are soluble in water, e.g. ethyl sulphuric acid, ethyl oxalic acid, etc.

Neutral esters are soluble in alcohol and ether, acid esters may be soluble in alcohol, but are insoluble in ether.
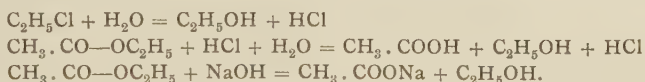
Isomerism occurs amongst the esters, e.g.

$CH_3 . COOCH_3$ is isomeric with $HCOOC_2H_5$
Methyl acetate.   Ethyl formate.
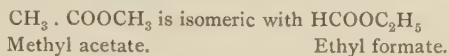
Esters are comparatively inert substances and are unaffected by cold dilute sodium carbonate, sodium hydroxide, hydrochloric acid, sulphuric acid, but there are exceptions, e.g. methyl oxalate, which is decomposed by cold dilute caustic soda. They are acted upon by sodium more or less readily (cf. ethers).

HYDROLYSIS OF ESTERS.

All esters are hydrolysed by boiling with water, acids, or alkalies. In the last case the sodium salt of the acid is formed. This method of hydrolysis is known as saponification. Esters are thus converted into their constituents, namely acid and alcohol. The recognition of these identifies the ester :—

$C_2H_5Cl + H_2O = C_2H_5OH + HCl$
$CH_3 . CO—OC_2H_5 + HCl + H_2O = CH_3 . COOH + C_2H_5OH + HCl$
$CH_3 . CO—OC_2H_5 + NaOH = CH_3 . COONa + C_2H_5OH.$

Esters which are of frequent occurrence in animals and plants are identified in this way.

The hydrolysis of esters is effected by boiling under a reflux condenser with aqueous sodium hydroxide, or 80 per cent. sulphuric acid.

If only the acid is to be identified, hydrolysis is effected by boiling with alcoholic sodium hydroxide, distilling off the alcohol, adding water and acidifying the solution.

The alcohol is isolated by distilling the alkaline liquid if the alcohol be volatile, by extracting the alkaline solution with ether if not volatile, and it is identified by the reactions for alcohols.

If the alcoholic portion of the ester be a phenol, or an aromatic alcohol, it does not distil and is not extracted from an alkaline solution. The solution must be firstly acidified to liberate the phenol (see under phenols).
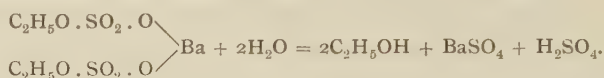
The acid, which is formed by hydrolysis with alkali, is liberated by acidifying the cold solution with mineral acid—sulphuric acid. If insoluble it is filtered off; if soluble and volatile with steam, distilled ; if soluble and not volatile, it is extracted with ether, or precipitated as insoluble calcium or other salt.

* **Ethyl Sulphuric Acid.**

Ethyl sulphuric acid is not readily hydrolysed by alkali, but it is decomposed by boiling with acid.

The solution of calcium, or barium, ethyl sulphate obtained above contains one or other of these bases as shown by adding dilute sulphuric acid, the insoluble sulphate being precipitated.

If a portion of the solution be heated with dilute hydrochloric acid for 3-5 minutes, the insoluble sulphate is again precipitated :—

$$\begin{array}{l} C_2H_5O.SO_2.O \\ \hspace{-1.5em}\Big\rangle Ba + 2H_2O = 2C_2H_5OH + BaSO_4 + H_2SO_4. \\ C_2H_5O.SO_2.O \end{array}$$

* **Ethyl Acetate.**

About 10 c.c. of ethyl acetate are placed in a flask with about 80 c.c. of sodium hydroxide, and the mixture is boiled over a gauze under a reflux condenser for 20-30 minutes until no more oily drops are visible and until the smell of ethyl acetate has disappeared.   A piece of unglazed porcelain is added with advantage to prevent bumping of the liquid during the heating :—

$$CH_3COOC_2H_5 + NaOH = CH_3COONa + HOC_2H_5.$$
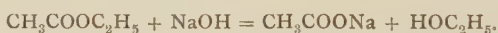
The required quantity of sodium hydroxide for the saponification is calculated from this equation.

The flask is connected with a condenser and about a quarter of the liquid is distilled over.

This liquid contains the alcohol.   It may be identified by the tests for ethyl alcohol.

The alcohol is separated and identified by saturating the solution with solid potassium carbonate, collecting the alcohol in a pipette, determining its boiling-point and performing other reactions for the alcohol.

The liquid remaining in the flask is acidified with dilute sulphuric acid and again distilled as long as the distillate reacts acid to litmus.

The distillate is neutralised and evaporated down and the acetic acid prepared and identified (p. 97).

* **Ethyl Benzoate.**

The hydrolysis is effected as described above for ethyl acetate.

The insoluble acid is more readily prepared by hydrolysing with alcoholic soda and then identified :

5-10 gm. of the ester are placed in a flask and boiled under a reflux condenser with excess of caustic soda (1-3 gm.), dissolved in 10 c.c.

water and 100 c.c. of alcohol, for 10-15 minutes. The saponification is continued until a few drops poured into water show no oily drops of unchanged ester.

If any insoluble sodium benzoate separates out, it is dissolved by adding a little water through the condenser.

The solution is poured into an evaporating basin, water added and the alcohol evaporated off on the water-bath. On cooling and after adding about 25-50 c.c. of water, the solution is acidified with dilute mineral acid. Benzoic acid is precipitated. It is washed with water, recrystallised from hot water, and identified (p. 198).

Ethyl Oxalate.

10-20 gm. of ethyl oxalate are hydrolysed with caustic soda solution containing sufficient alkali (6-12 gm.) as described under ethyl acetate and the alcohol is distilled off.

The acid contained in the solution on acidifying with mineral acid is not precipitated, nor is it volatile with steam. The acid solution may be extracted several times with ether, the ethereal solution distilled to remove the ether, and the acid which is left identified.

Oxalic acid is more easily separated as its calcium salt. The acid liquid is carefully neutralised with soda and calcium oxalate is precipitated by adding calcium chloride. The acid is obtained as described under oxalic acid (p. 139).
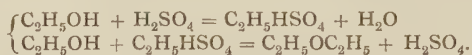
CHAPTER VII.

ETHERS.

ETHER was first made by distilling alcohol with concentrated sulphuric acid :—

$$2C_2H_5OH + H_2SO_4 = (C_2H_5)_2O + H_2O + H_2SO_4.$$
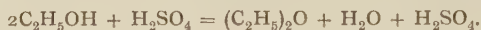
The reaction thus appears to be one of removal of one molecule of water from two molecules of alcohol, but in reality it is more complicated. Ethyl sulphuric acid is first formed and is decomposed by alcohol :—

$$\begin{cases} C_2H_5OH + H_2SO_4 = C_2H_5HSO_4 + H_2O \\ C_2H_5OH + C_2H_5HSO_4 = C_2H_5OC_2H_5 + H_2SO_4. \end{cases}$$

This explanation of the reaction was only given after the discovery of the constitution of ether by Williamson in 1851. Ether was made by the action of ethyl iodide upon sodium ethoxide, two compounds whose constitution was known, thus

$$C_2H_5ONa + C_2H_5I = C_2H_5OC_2H_5 + NaI.$$

Ether is thus composed of two ethyl groups joined to oxygen. The extension of this reaction to other alkyl iodides and sodium derivatives of other alcohols (sodium alkoxides) confirmed this constitution. The same ether is given by the two reactions :—

$$CH_3ONa + C_2H_5I = CH_3OC_2H_5 + NaI$$
$$C_2H_5ONa + CH_3I = C_2H_5OCH_3 + NaI.$$
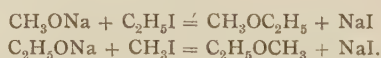
An ether of this type containing two different alkyl radicles is known as a mixed ether in contrast with ordinary ether, or dimethyl ether, which is a simple ether ; the two radicles in a simple ether are the same. The proof that the reaction of alcohol and sulphuric acid takes place in two stages was given by the preparation of the same mixed ether by using a different alcohol in the second reaction, thus

$$CH_3OH + C_2H_5HSO_4 = CH_3OC_2H_5 + H_2SO_4$$
$$C_2H_5OH + CH_3HSO_4 = C_2H_5OCH_3 + H_2SO_4.$$
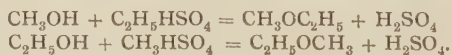
Ordinary ethyl ether is the only ether of importance.

ETHYL ETHER.

Preparation.

Ethyl ether is generally prepared by distilling ethyl alcohol with sulphuric acid—hence its old name of sulphuric ether. According to the

equation the sulphuric acid is combined and again liberated so that it should be possible to convert an unlimited quantity of alcohol into ether, but by-products are formed which interfere with the reaction and fresh sulphuric acid has to be added from time to time. Some of the sulphuric acid is reduced to SO_2 by the organic matter and it is lost in this way. The process is known as the continuous process.

A distilling flask of about 500 c.c. capacity is fitted with a tap funnel and a thermometer, the bulb of which reaches nearly to the bottom of the flask. The neck of the flask is connected to a long condenser and the receiver is cooled by standing in ice water. A mixture of 110 c.c. of absolute alcohol and 80 c.c. of concentrated sulphuric acid is placed in the flask and heated to 140-145°. At this temperature ether is formed and absolute alcohol is dropped in from the tap funnel at the same rate as the liquid distils. The preparation is continued until about twice the volume of alcohol originally mixed with the sulphuric acid has been added. The distillate consists of ether, alcohol, water and sulphurous acid. It is put into a separating funnel and shaken with dilute caustic soda. The alkaline layer is withdrawn and the upper layer of ether shaken with saturated salt solution, which is also withdrawn. The ether is put into a distilling flask, which is loosely corked, and dried by being allowed to stand in contact with calcium chloride for 12-24 hours. The flask is connected with a condenser and the ether distilled off from a water-bath (b.p. 35°).

Purification of Ethyl Ether.

The ether obtained above contains traces of alcohol and water. These can only be removed by treatment with metallic sodium. The ether is placed in a flask, provided with a calcium chloride tube to prevent access of moisture and to allow the escape of hydrogen, and several slices of sodium are added. When no further effervescence is observed the ether is decanted into a distilling flask and distilled from a water-bath. Pure ether of constant boiling-point 35° is collected.

Purification of Commercial Methylated Ether.

This ether is made by the continuous process from methylated spirit and contains water, alcohol and other impurities. The ether may be washed with water to remove most of the alcohol. By distilling it over solid caustic potash, aldehydic impurities are destroyed. It is dried by standing over calcium chloride and then treated with metallic sodium.

Sometimes, after treatment with sodium, ether is left in contact with phosphorus pentoxide and then distilled from the solid dehydrating agent.

* Distillation of Ether. Precautions.

As ether is very inflammable and exceedingly volatile, no flame should be in the neighbourhood. Ether should never be distilled over a free flame and the most convenient way, if steam or electric heaters are not available, is to heat a water-bath, extinguish the flame, and immerse the distilling flask containing the ether in the hot water.

Large quantities of ether should not be distilled from a large flask, but a small flask provided with a tap funnel should be employed. As

the ether distils a fresh quantity can be added without interrupting the distillation.   The ether should be collected in small receivers and transferred to a larger reservoir.

Properties.

The first member of the series, dimethyl ether, is a gas.

Ethyl ether, or simply ether, the chief representative of the group, is a very volatile, colourless liquid with a pleasant characteristic smell. It boils at 35° and has a sp. gr. of ·7195 at 15°.   It is sparingly soluble in water, less soluble in glycerol.   It mixes in all proportions with alcohol, chloroform, benzene, ligroin, and is largely used as a solvent for fats, resins, etc.

The lower members of ethers of the aliphatic series are also volatile liquids, like ethyl ether, which boil at a lower temperature than the alcohol from which they are derived.   The highest members are odourless solids.

The ethers are inert compounds and are not acted upon by phosphorus pentachloride and sodium (distinction from alcohols), or by aqueous or alcoholic potash (distinction from halogen compounds and esters).

The lower members—especially those containing methyl and ethyl radicles—are decomposed by heating with hydriodic acid forming alkyl iodides (distinction from hydrocarbons) :—

$$C_2H_5OC_2H_5 + 2HI = 2C_2H_5I + H_2O$$
$$CH_3OC_2H_5 + 2HI = CH_3I + C_2H_5I + H_2O.$$
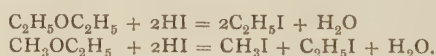
This reaction is used in estimating methoxy—CH_3O—and ethoxy C_2H_5O groups in organic compounds (Zeisel's method).

It should be noticed that certain alcohols and ethers are isomers :—

C_2H_5OH	CH_3OCH_3
C_3H_7OH	$CH_3OC_2H_5$
C_4H_9OH	$C_2H_5OC_2H_5$
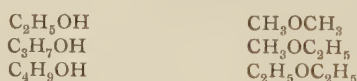
CHAPTER VIII.

MERCAPTANS AND SULPHIDES.

The sulphur compounds corresponding to the alcohols, i.e. thio-alcohols, are known as mercaptans ; the sulphur compounds corresponding to the ethers, i.e. thio-ethers, are known as sulphides, or alkyl sulphides. Disulphides are also known.

$$CH_3 . SH \qquad C_2H_5 . S . C_2H_5 \qquad C_2H_5 . S—S . C_2H_5$$
Methyl mercaptan.   Ethyl sulphide.   Ethyl disulphide.

Mercaptans.

Methyl mercaptan is a product of the putrefaction of proteins. It occurs in the urine after a diet of asparagus and gives it the peculiar unpleasant odour.

Preparation.

Mercaptans are prepared :—

(1) By heating the alcohol with phosphorus pentasulphide :—

$$5CH_3OH + P_2S_5 = 5CH_3SH + P_2O_5.$$
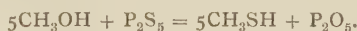
(2) By heating the alkyl halide, or alkyl potassium sulphate, with potassium hydrosulphide :—

$$CH_3I + KSH = CH_3SH + KI$$
$$C_2H_5O . SO_2 . ONa + KSH = C_2H_5SH + NaKSO_4.$$

About 2-5 c.c. of a saturated solution of sodium ethyl sulphate are made alkaline with sodium hydroxide and an equal volume of sodium hydrosulphide (33 per cent.) is added. On warming, ethyl mercaptan is formed which is recognised by its garlic-like unpleasant odour.

Properties.

Methyl mercaptan is a gas, ethyl mercaptan is a colourless liquid boiling at 36°. The other mercaptans are also liquids which are insoluble in water and have a disgusting smell.

Like the alcohols they react with sodium with evolution of hydrogen :—

$$2CH_3SH + Na_2 = 2CH_3SNa + H_2.$$
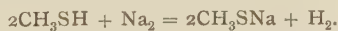
The mercaptans react with mercuric oxide forming crystalline compounds :—

$$2C_2H_5SH + HgO = (C_2H_5 . S)_2Hg + H_2O.$$
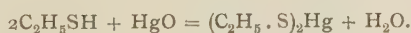
These compounds are termed mercaptides, the name of the group being derived from the mercury compounds.
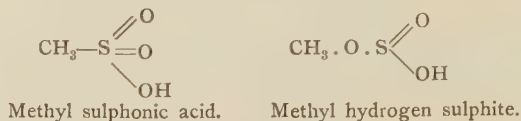
Sulphonic Acids.

On oxidation with nitric acid the mercaptans yield sulphonic acids :—

$$CH_3SH + 3O = CH_3 . SO_3H.$$
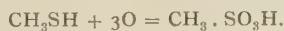
These are strong monobasic acids, soluble in water and forming salts with metallic oxides. Alkali salts of the sulphonic acids are formed by the action of the alkyl halide on potassium sulphite :—

$$C_2H_5I + K_2SO_3 = C_2H_5SO_3K + KI.$$
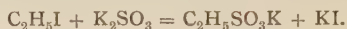
The sulphonic acids are isomeric with alkyl hydrogen sulphites. The latter compounds are esters and are hydrolysed by alkali; the sulphonic acids are stable. In the sulphonic acids the sulphur atom is joined to carbon, in the sulphites it is joined to oxygen :—

$$CH_3{-}S{\nwarrow}{\genfrac{}{}{0pt}{}{O}{O}}{\searrow}{OH} \qquad CH_3.O.S{\nwarrow}{\genfrac{}{}{0pt}{}{O}{}}{\searrow}{OH}$$

Methyl sulphonic acid.   Methyl hydrogen sulphite.

Alkyl Sulphides, or Thio-Ethers.

Ethyl sulphide, $C_2H_5.S.C_2H_5$, is another product of the putrefaction of proteins, being derived from cystine (p. 174).

Preparation.

Sulphides are obtained :—

(1) By the action of phosphorus pentasulphide upon ethers :—

$$5(C_2H_5)_2O + P_2S_5 = 5(C_2H_5)_2S + P_2O_5.$$

(2) By the action of potassium sulphide on an alkyl halide, or alkyl potassium sulphate :—

$$2C_2H_5I + K_2S = 2KI + (C_2H_5)_2S$$
$$2C_2H_5KSO_4 + K_2S = 2K_2SO_4 + (C_2H_5)_2S.$$
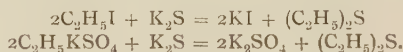
Properties.

The sulphides are colourless, neutral liquids with very unpleasant smell; ethyl sulphide boils at 91°.

They resemble the ethers in being comparatively stable compounds. On oxidation with nitric acid, they are converted into sulphones which are stable crystalline compounds :—

$$(C_2H_5)_2S + O_2 = (C_2H_5)_2SO_2.$$
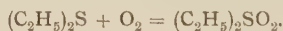
Alkyl Disulphides.

Disulphides are formed when mercaptans are exposed to the air :—

$$2C_2H_5SH + O = H_2O + C_2H_5.S{-}S.C_2H_5,$$
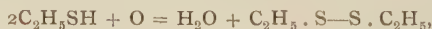
or by the action of iodine upon sodium mercaptides :—

$$2C_2H_5S.Na + I_2 = 2NaI + C_2H_5.S{-}S.C_2H_5.$$

The oxidation of the thio-alcohol to the disulphide is a reaction of great importance in the function of the living cell, and occurs with the compounds cysteine and cystine, more especially in their combination product with glutamic acid, known as glutathione.

CHAPTER IX.

ALDEHYDES are the first products of oxidation of primary alcohols:—

$$CH_4O + O = CH_2O + H_2O$$
$$C_2H_6O + O = C_2H_4O + H_2O.$$
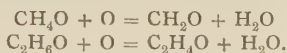
The reaction is one of *dehydrogenation*. The alcohol loses two atoms of hydrogen, which combine with an atom of oxygen. The oxygen may be regarded as a *hydrogen acceptor*.

The reaction may be considered as an oxidation, by imagining one of the hydrogen atoms of the —CH_2OH group, but not OH as becoming oxidised to OH forming a hypothetical intermediate compound with two OH groups. This compound does not exist; it at once loses a molecule of water and is converted into aldehyde:—

Ethyl alcohol.   Hypothetical.   Acetaldehyde.
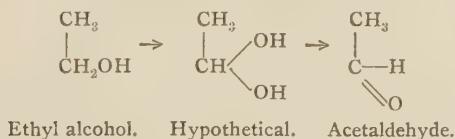
Generally, two OH groups cannot exist attached to one carbon atom, but there are a few exceptions, such as chloral hydrate,
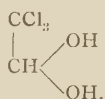
Formaldehyde is formed from methyl alcohol, propyl aldehyde from primary propyl alcohol, etc.,
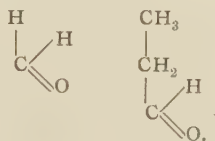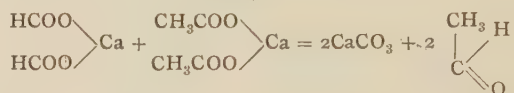

The group —CHO or —$C\diagdown^{H}_{O}$ is characteristic of aldehydes; it is situated at the end of a chain of carbon atoms. Aldehydes are named after the acid to which they give rise on oxidation, or after the primary

alcohol from which they originate.   The suffix-al is used to designate them, e.g. ethanal.
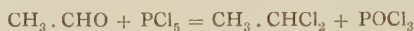
Preparation.

When the alcohol is available, the aldehyde is usually prepared from it by oxidation; otherwise, it may be prepared by the dry distillation of molecular proportions of calcium formate and the calcium salt of the corresponding acid (compare ketones).

$$\begin{matrix} HCOO \\ \\ HCOO \end{matrix} \!\!\!\Big\rangle Ca + \begin{matrix} CH_3COO \\ \\ CH_3COO \end{matrix} \!\!\!\Big\rangle Ca = 2CaCO_3 + 2 \begin{matrix} CH_3 \;\; H \\ \big| \;\; \diagup \\ C \\ \diagdown O. \end{matrix}$$

Constitution.

The formation of aldehydes from primary alcohols by oxidation and the reaction of aldehydes with phosphorus pentachloride :—

$$CH_3 . CHO + PCl_5 = CH_3 . CHCl_2 + POCl_3$$

in which the oxygen is replaced by two atoms of chlorine shows that they contain an O atom joined to a carbon atom.   (Cf. alcohol and phosphorus pentachloride.)

FORMALDEHYDE.

Preparation.

Formaldehyde is prepared by passing the vapour of methyl alcohol mixed with air over heated platinum or copper, or other substances. The formaldehyde formed by oxidation is passed into water.

It is produced by the reduction of carbon monoxide by hydrogen in presence of a metallic catalyst

$$H_2 + CO = CH_2O.$$
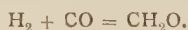
This reaction is reversible.

ACETALDEHYDE.

Preparation.

25 gm. of coarsely powdered potassium bichromate and 100 c.c. of water are placed in a distilling flask of 250 c.c. capacity.   The flask is connected with a condenser and a strong current of cold water is made to flow through it.   Through a tap funnel, secured in the neck of the flask by a well-fitting cork, a mixture of 25 gm. (30 c.c.) of absolute alcohol and 35 gm. (20 c.c.) of concentrated sulphuric acid is slowly added to the contents of the flask which have been gently warmed and the flame removed.   During the addition the contents of the flask, which darken in colour, are occasionally shaken.   A mixture of alde-

hyde, alcohol, and water distils over. When the mixture of alcohol and sulphuric acid has been added, the flask is heated until all the aldehyde (recognised by smell) has distilled over.

The tests for acetaldehyde can be carried out with this distillate (p. 82).

Purification.

The solution is redistilled through an inverted condenser filled with water at 30-35°. Water and alcohol are condensed, but the aldehyde passes on. The aldehyde vapour is passed through a 100 c.c. pipette into about 30 c.c. of pure dry ether contained in a bottle standing in ice.

Pure ammonia, prepared by gently heating concentrated ammonia solution with a small flame and dried by passage through a tower containing quick-lime, is passed into the ether until it is saturated. Aldehyde ammonia crystallises out. After standing for one hour the ether is decanted off, the crystals are drained on a Buchner filter and washed with ether.

The crystals of aldehyde ammonia are dissolved in an equal weight of water, and the solution is distilled with a mixture of 1·5 parts of sulphuric acid and 2 parts of water from a water-bath, which is gradually raised to boiling. The receiver is cooled in ice. The distillate is dried with calcium chloride from which the aldehyde is distilled in a bath at 20° and collected in a receiver in ice. The aldehyde must be preserved in a well-stoppered bottle.

PROPERTIES OF ALDEHYDE.

Formaldehyde is a gas at the ordinary temperature, easily soluble in water and alcohol and with a peculiar pungent smell.

Formaldehyde is a powerful antiseptic (prevents growth of micro-organisms) and disinfectant (destroys micro-organisms). Formalin, or solid paraform, is commonly used for these purposes. A drop of formalin in a pint of milk will keep the milk sweet for many days. The use of formaldehyde for purposes of food preservation is not allowed. Rooms are disinfected, after illness, by heating formalin, or paraform, on a tray over a spirit lamp. Formaldehyde volatilises and fills the room with disinfectant vapour.

Formol, or formalin, is a commercial aqueous solution containing 40 per cent. of formaldehyde.

Acetaldehyde is a colourless liquid having a fruity pungent smell and boils at 21°. It is easily soluble in water, alcohol, and ether.

The next members of the series of aldehydes are also liquids and resemble acetaldehyde very closely in their properties. On reduction with zinc and acetic acid or sodium amalgam, they give primary alcohols.
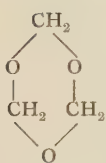
On oxidation, aldehydes yield an acid with the same number of carbon atoms (see fatty acids).

Polymerisation.

The formation of a compound of the same composition, but of higher molecular weight is known as polymerisation. A polymer is easily re-converted into its original simple molecules.

Paraformaldehyde or Paraform, $(CH_2O)_n$.

*

$$CH_2$$
$$O \quad O$$
$$CH_2 \quad CH_2$$
$$O$$

On evaporating formalin (about 1 c.c.) in a watch-glass on a water-bath, a solid mass of paraformaldehyde is left.

If a portion of the solid be heated in a test tube, dissociation occurs at about 100°, the mass melts between 153 and 172°, formaldehyde is evolved, and a white sublimate of trioxymethylene is formed.

Paracetaldehyde or Paraldehyde, $(CH_3 . CHO)_3$.

*

$$CH_3$$
$$CH$$
$$O \quad O$$
$$CH_3—CH \quad CH—CH_3$$
$$O$$
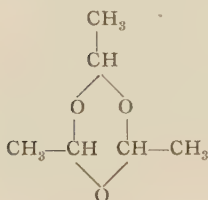
On adding a drop of concentrated sulphuric acid to about 1 c.c. of acetaldehyde, a violent reaction occurs and the liquid becomes hot. Paraldehyde separates out as an oil on diluting with water. Acetaldehyde is re-formed on distilling it with dilute sulphuric acid.

Paraldehyde is a colourless liquid which boils at 124°. It is used in medicine as a soporific.

Metaldehyde.

Another polymer, metaldehyde, is formed from acetaldehyde when it is treated with hydrochloric acid gas, or dilute sulphuric acid at a low temperature.

Aldol Condensation.

A solution of formaldehyde when kept with dilute solutions of lime, or potassium carbonate, undergoes aldol condensation.

Two and more molecules combine :—

$$HCHO + HCHO = HCHOH . CHO$$
$$HCHO + HCHO + HCHO = HCHOH . CHOH . CHO$$
$$6CH_2O = C_6H_{12}O_6$$
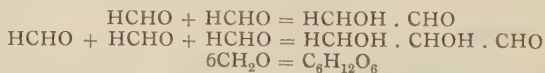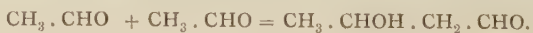
A sweet syrup which contains monosaccharides, especially dl-fructose is the final result.

This reaction of formaldehyde probably takes place in nature. In plants, under the action of light and chlorophyll, carbon dioxide is reduced to formaldehyde which undergoes aldol condensation into sugars.

Acetaldehyde gives aldol :—

$$CH_3 . CHO + CH_3 . CHO = CH_3 . CHOH . CH_2 . CHO.$$

Aldol easily changes into crotonaldehyde. The higher fatty acids are probably formed from acetaldehyde in this way in both animals and plants.
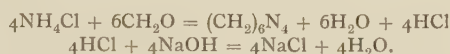
Action of Ammonia.

Hexamethylene Tetramine.

Formaldehyde behaves differently to the other aldehydes.

* On adding ammonia gradually to formalin (1 c.c. in 5 c.c. water) it is absorbed. On now adding excess of ammonia and evaporating on the water-bath hexamethylene tetramine, or urotropin, remains as a white solid :—

$$6CH_2O + 4NH_3 = (CH_2)_6N_4 + 6H_2O.$$
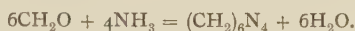
This reaction serves for estimating ammonia in its salts :—

$$4NH_4Cl + 6CH_2O = (CH_2)_6N_4 + 6H_2O + 4HCl$$
$$4HCl + 4NaOH = 4NaCl + 4H_2O.$$

The free acid which is liberated is titrated with standard alkali.

Hexamethylene tetramine consists of colourless crystals soluble in about 1·5 parts of hot or cold water and 10 parts of alcohol. It is volatilised on heating and it is converted into ammonium sulphate and formaldehyde on treatment with strong sulphuric acid.

Aldehyde Ammonia.

On passing dry ammonia gas into a dry ethereal solution of acetaldehyde, acetaldehyde ammonia is formed :—

$$CH_3.CHO + NH_3 = CH_3.CH{\Large\langle}^{OH}_{NH_2.}$$
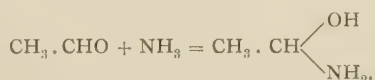
Acetaldehyde ammonia is a white crystalline compound easily soluble in water and alcohol, and easily decomposed by acids and alkalies.

* On dissolving a little aldehyde ammonia and heating with dilute sulphuric acid, aldehyde is given off. Ammonia is also evolved on heating with dilute caustic soda.

Aldehyde Sodium Bisulphite.

* On adding 1-2 c.c. of a cold saturated solution of sodium bisulphite to 5-10 drops of aldehyde and shaking vigorously, aldehyde sodium bisulphite crystallises out :—

$$CH_3.CHO + NaHSO_3 = CH_3.CH{\Large\langle}^{OH}_{SO_3Na}$$
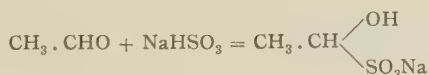
Aldehyde Cyanhydrin.

Hydrogen cyanide combines with aldehydes forming cyano-hydrins :—

$$CH_3 . CHO + HCN = CH_3 \cdot CH{<}^{OH}_{CN.}$$

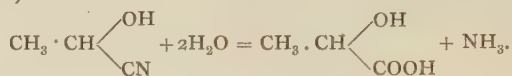In this way another carbon atom can be added to organic compounds. Compounds containing the CN group are hydrolysed by acids or alkalies and converted into the corresponding acid (see cyanogen compounds) :—
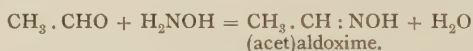
$$CH_3 \cdot CH{<}^{OH}_{CN} + 2H_2O = CH_3 . CH{<}^{OH}_{COOH} + NH_3.$$

Aldehyde Hydrazone.

Aldehydes combine with hydrazine and substituted hydrazines, especially phenylhydrazine, forming hydrazones.

* The calculated quantities of aldehyde (·5 c.c.), phenylhydrazine hydrochloride (·2 gm.) and cryst. sodium acetate (·5 gm.) are dissolved in about 10 c.c. of water and warmed ; an oil (acetaldehyde phenyl-hydrazone) is formed :—

$$CH_3 . CHO + H_2N . NH . C_6H_5 = CH_3 . CH : N . NH . C_6H_5 + H_2O.$$
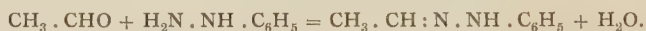
Aldoxime.

Aldehydes combine with hydroxylamine forming oximes :—

$$CH_3 . CHO + H_2NOH = CH_3 . CH : NOH + H_2O$$
(acet)aldoxime.

The calculated quantity of hydroxylamine hydrochloride is dissolved in water, the equivalent quantity of caustic soda required to liberate the hydroxylamine is added and then the calculated quantity of the aldehyde. The mixture is shaken and allowed to stand until it no longer reduces Fehling's solution. The oxime is extracted with ether, most of the ether distilled off, and the concentrated solution poured into a basin. The crystals which separate are drained on a porous plate and recrystallised from ligroin.

Tests.

Aldehydes are easily further oxidised into the corresponding fatty acids containing the same number of carbon atoms and they consequently behave as reducing agents.

Reduction of Metallic Oxides in Alkaline Solution.

(*a*) *Silver.*

* An ammoniacal solution of silver hydroxide is prepared by adding dilute ammonia to silver nitrate until the precipitate first formed just re-dissolves. Some dilute aldehyde solution is added and the mixture

is placed in a cold water-bath and heated to the boiling-point. A mirror of metallic silver forms on the glass.

A very sensitive reagent may be prepared by mixing equal volumes of 10 per cent. silver nitrate and sodium hydroxide and then adding ammonia drop by drop till the silver hydroxide dissolves.

A mirror is formed immediately if the solution contains 1 per cent. of acetaldehyde, in 30 seconds if 1 per thousand : a yellow-brown mirror forms in 5 minutes if 1 per 10,000 be present.

(b) *Copper.*

* Dilute aldehyde solution reduces Fehling's solution[1] on warming with the formation of cuprous oxide.

Action of Sodium Hydroxide.

* Except with formaldehyde, benzaldehyde and a few other aldehydes, caustic soda solution decomposes dilute aldehyde solutions on warming. Yellow to brownish-red resins which rise to the surface— *aldehyde resin*—are formed. The liquid has usually a peculiar smell. Aldehyde resin is insoluble in water, but soluble in alcohol and ether.

Formaldehyde is converted into methyl alcohol and formic acid.

Oxidation.

Aldehydes are converted into the corresponding acid on warming their solutions with potassium bichromate and dilute sulphuric acid, the solution becoming green.

The aldehyde may be identified by preparing the acid by oxidation.

Schiff's Test.

* A solution of magenta, or fuchsin, is decolorised by bubbling sulphur dioxide through it. On adding a dilute aldehyde solution, the purple-red colour returns.

Numerous other sensitive tests have been described for aldehydes, especially formaldehyde. The following one has been used more particularly in testing for formaldehyde in distillates from plant leaves, etc.

Rimini's Test.

A small quantity (2 drops) of phenylhydrazine is added to the solution, then a drop of dilute freshly prepared sodium nitroprusside solution and a few drops of sodium hydroxide solution. A deep blue colour forms if formaldehyde be present ; the colour changes through green and brown to red.

---

[1] Fehling's solution consists of copper sulphate, caustic soda, and Rochelle salt (sodium potassium tartrate). On adding caustic soda to copper sulphate a blue precipitate of cupric hydrate $Cu(OH)_2$ is formed, which turns black on boiling. The presence of the Rochelle salt keeps the $Cu(OH)_2$ in solution forming a deep blue solution. This solution does not keep, so that it must be freshly made for each experiment. For this purpose two solutions are therefore kept. The one contains the copper sulphate, the other the Rochelle salt and caustic soda. When required for use, equal parts of each are mixed together, and this forms the reagent.
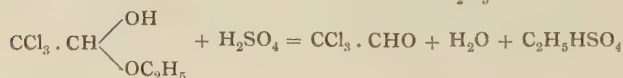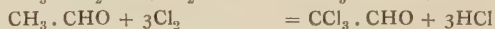
Schryver has modified this test and made it more sensitive: 2 c.c. of a freshly prepared and filtered 1 per cent solution of phenylhydrazine hydrochloride are added to 10 c.c. of the solution of formaldehyde, then 1 c.c. of a 5 per cent. solution of sodium ferricyanide, and 5 c.c. of hydrochloric acid ; a magenta colour is formed. This test will show the presence of 1 part of formaldehyde in 100,000 to 1,000,000 parts of solution. No colour is given by acetaldehyde.

$$\textbf{CHLORAL,} \quad \begin{array}{c} CCl_3 \\ | \\ CHO, \end{array} \quad \text{or Trichloracetaldehyde.}$$
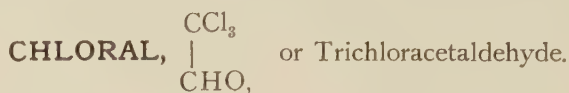
Preparation.

Chloral is prepared by the prolonged action (about 10 days) of dry chlorine upon absolute alcohol. The gas is passed into the cold alcohol until it is saturated and acquires a sp. gr. of 1·400 and the temperature is gradually raised to 100.° Chloral alcoholate crystallises out on cooling. The crystals are treated with concentrated sulphuric acid and the oil which separates is distilled. The fraction passing over between 94 and 100° is collected, neutralised with calcium carbonate and again distilled.

The reactions which occur are :—

$$CH_3 . CH_2OH + Cl_2 = CH_3 . CHO + 2HCl$$
$$CH_3 . CHO + 3Cl_2 = CCl_3 . CHO + 3HCl$$
$$CCl_3 . CHO + C_2H_5OH = CCl_3 . CH{\overset{\displaystyle OH}{\underset{\displaystyle OC_2H_5}{<}}}$$
$$CCl_3 . CH{\overset{\displaystyle OH}{\underset{\displaystyle OC_2H_5}{<}}} + H_2SO_4 = CCl_3 . CHO + H_2O + C_2H_5HSO_4$$

Properties.

Chloral is a colourless oily liquid with a peculiar penetrating smell, having a sp. gr. of 1·502 at 18°. It boils at 97° and is soluble in ether and chloroform. It combines with water giving chloral hydrate (below).

Metachloral.

On keeping, or on leaving in contact with moderately concentrated sulphuric acid, chloral polymerises to metachloral, a solid which is sparingly soluble in boiling water, but insoluble in cold water, alcohol, and ether. The polymerisation does not occur with pure chloral and may be hindered by adding chloroform. On heating to 180° meta-chloral is decomposed and chloral distils over.

$$\textit{Chloral Alcoholate,} \quad CCl_3 . CH{\overset{\displaystyle OH}{\underset{\displaystyle OC_2H_5}{<}}}.$$

If chloral be mixed with an equivalent quantity of absolute alcohol, chloral alcoholate is formed.

It consists of white crystals which melt at $46°$ and boil at $113\cdot5°$ and are readily soluble in chloroform (distinction from chloral hydrate).

CHLORAL HYDRATE, $CCl_3 . CH\langle\begin{smallmatrix}OH\\OH\end{smallmatrix}$.

Preparation.

Equivalent parts of chloral (6 c.c.) and water (1 c.c.) are mixed together. The mixture becomes hot and solidifies to a mass of crystals of chloral hydrate.

Properties.

Chloral hydrate is a white crystalline solid, which melts at $50\text{-}51°$. It is soluble in $1\cdot5$ times its weight of water, also in alcohol, ether, petroleum ether, and carbon disulphide. It is soluble with difficulty in cold chloroform.

Pure chloral hydrate is completely volatile on heating and commences to boil rapidly at $97\text{-}98°$.

It finds use in medicine as a soporific. In the body chloral is reduced to trichlorethyl alcohol which is excreted in the urine as urochloralic acid, a combination product with glycuronic acid.

Reconversion into Chloral.

About 2 gm. of chloral hydrate are placed in a dry test tube and covered with concentrated sulphuric acid and the mixture is warmed gently. Chloral is formed and floats to the surface.

Tests for Chloral and Chloral Hydrate.

Aqueous solutions in the cold give no reaction with silver nitrate. On adding a few drops of ammonia and boiling, metallic silver is deposited.

Aqueous solutions reduce Fehling's solution on heating. Traces of chloral may be detected by the carbylamine reaction for chloroform (p. 54).

Decomposition of Chloral by Alkali.

Chloral, or chloral hydrate, is rapidly decomposed by caustic alkali with the formation of chloroform and alkali formate :—

$$CCl_3CH(OH)_2 + NaOH = CHCl_3 + HCOONa + H_2O.$$
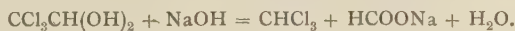
The odour of chloroform is noticed at once on gently warming an aqueous solution of chloral with caustic soda.

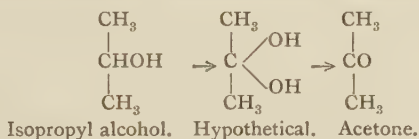### Butyric Chloral Hydrate.

This compound is formed when chlorine is passed into paraldehyde or acetaldehyde. It is a white crystalline substance with peculiar fruity flavour and melts at 78°.

CHAPTER X.

KETONES are the first products of the oxidation of secondary alcohols. The same statements apply here as in the case of the formation of aldehydes. The reaction is one of dehydrogenation. Oxidation can be imagined to occur through a hypothetical intermediate compound :—

$$
\begin{array}{ccc}
CH_3 & CH_3 & CH_3 \\
| & | \quad OH & | \\
CHOH & \rightarrow C & \rightarrow CO \\
| & | \quad OH & | \\
CH_3 & CH_3 & CH_3 \\
\end{array}
$$

Isopropyl alcohol.   Hypothetical.   Acetone.

The group $>CO$ is characteristic of ketones.

Acetone is the first member of the homologous series of ketones and the chief representative. Either the suffix -one is used to designate ketones, or the term keto.

Ketones, on reduction, yield the corresponding secondary alcohol. On oxidation, the molecule breaks at the $>CO$ grouping and two acids with fewer carbon atoms in the molecule are produced (p. 89).

The constitution of a ketone is determined by identifying the acids it yields on oxidation.

From 2-5 gm. of the ketone are mixed in a flask attached to a reflux condenser with 30-50 c.c. water and the calculated quantity of sulphuric acid is added. The calculated quantity of finely powdered potassium bichromate is added in portions of ·5-1 gm. If the oxidation is very energetic, the contents should be cooled and kept at 50-60°. The flask is finally heated on the water-bath for 15 minutes. The acids are then distilled and collected in the receiver (see under acids).

ACETONE.

Acetone occurs in traces in normal urine, but in diabetes the amount is largely increased. It is formed by decomposition of aceto-acetic acid (p. 157). Its detection in urine and estimation is of great practical importance in medicine.

Preparation.

Acetone is formed in the dry distillation of wood and is separated from methyl alcohol by fractional distillation (p. 59).

Acetone is also prepared by the dry distillation of calcium, or barium, acetate :—

$$\begin{matrix} CH_3.COO \\ \\ CH_3.COO \end{matrix}\!\!\Big\rangle Ca = \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\Big\rangle CO + CaCO_3.$$

* 50-100 gm. of dry calcium acetate are placed in a retort or distilling flask and at first heated gently, afterwards more strongly, and the vapours are passed through a condenser. A brownish liquid collects in the receiver. It contains acetone, aldehyde, and higher ketones. The acetone is separated by fractional distillation.

Purification.

The proper quantity of crude acetone (100 gm. or 125 c.c.) is added to the calculated quantity of sodium bisulphite (70 gm.) in saturated solution (this should smell of sulphur dioxide, if not, SO_2 is passed into it until it smells strongly of the gas), and the mixture is shaken vigorously in a closed vessel. Heat is evolved and a mass of crystals, $C_3H_6O . NaHSO_3$, separates out. After standing, the crystals are filtered off on a Buchner funnel and well drained. They are placed in a distilling flask and decomposed by adding a solution of sodium carbonate (40 gm.). The solution is distilled, preferably using a fractionating column, until the thermometer reaches 60°.

The distillate is dried with calcium chloride and the acetone distilled off.

Properties.

Ketones closely resemble aldehydes in most of their properties, but there are several differences.

Acetone is a colourless, pleasant smelling liquid which boils at 56° and has a sp. gr. of ·797 at 15°. It is very volatile and inflammable. It mixes with water, alcohol, and ether in all proportions. Like alcohol it can be separated from water by saturating the solution with potassium carbonate.

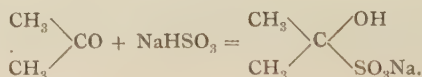### Polymerisation and Condensation.

Acetone does not polymerise like aldehyde, but when distilled with moderately concentrated sulphuric acid it is converted into mesitylene (sym. trimethylbenzene).

Action of Ammonia.

Acetone does not form simple condensation products with ammonia like aldehyde does, but it reacts forming diacetonamine, $C_6H_{13}ON$, and triacetonamine, $C_9H_{17}ON$.

Acetone Sodium Bisulphite.

On shaking together about 1 c.c. of acetone and 5 c.c. of a cold saturated solution of sodium bisulphite, acetone sodium bisulphite crystallises out :—
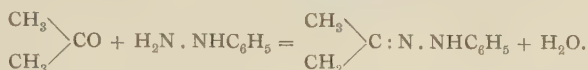
$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\Big\rangle CO + NaHSO_3 = \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\Big\rangle C\!\!\Big\langle\begin{matrix} OH \\ \\ SO_3Na. \end{matrix}$$

Acetone Cyanhydrin.

Acetone combines with hydrogen cyanide forming the addition compound, acetone cyanhydrin :—

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \Big\rangle CO + HCN = \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \Big\rangle C \begin{matrix} OH \\ \\ CN. \end{matrix}$$

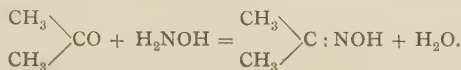## Acetone Phenylhydrazone.

Acetone combines with hydrazine and substituted hydrazines forming hydrazones :—

Acetone phenylhydrazone is formed as an oil when acetone is mixed with phenylhydrazine hydrochloride and sodium acetate :—

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \Big\rangle CO + H_2N . NHC_6H_5 = \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \Big\rangle C : N . NHC_6H_5 + H_2O.$$

Acetoxime.

Combination occurs between acetone and hydroxylamine when the calculated quantities are allowed to react together as described under aldehyde (p. 82) :—

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \Big\rangle CO + H_2NOH = \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \Big\rangle C : NOH + H_2O.$$

Tests for Acetone.

Acetone is more stable than aldehyde and does not behave as a reducing agent.

Acetone reduces ammoniacal silver nitrate solution on prolonged boiling.

Acetone does not reduce Fehling's solution.

Acetone does not give a resin when heated with sodium hydroxide.

Acetone does not give Schiff's test.

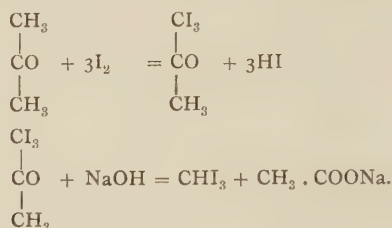These four reactions are characteristic only for aldehydes.

Oxidation.

Acetone is oxidised on heating with potassium bichromate and sulphuric acid and yields acetic and formic acids :—

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \Big\rangle CO + 3O = CH_3COOH + HCOOH.$$
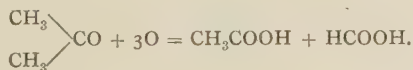
Iodoform Reaction (Lieben).

Acetone gives iodoform in the cold ; 3-5 drops of sodium hydroxide are added to about 2 c.c. of the solution and then, drop by drop,

iodine solution until the liquid is faintly yellow. Iodoform separates at once.

$$\begin{array}{ccc} CH_3 & & CI_3 \\ | & & | \\ CO & + 3I_2 & = CO & + 3HI \\ | & & | \\ CH_3 & & CH_3 \end{array}$$

$$\begin{array}{c} CI_3 \\ | \\ CO \\ | \\ CH_3 \end{array} + NaOH = CHI_3 + CH_3 . COONa.$$

If ammonia be used in place of sodium hydroxide and iodine solution be added drop by drop, a small black precipitate of nitrogen iodide is formed. On standing, or on warming, this disappears and iodoform is produced; this reaction may serve to distinguish acetone and alcohol.

*   *Sodium Nitroprusside Test (Legal).*

On adding about 5 drops of freshly prepared sodium nitroprusside solution to about 5 c.c. of the dilute acetone and about 1 c.c. of sodium hydroxide, a ruby-red colour is produced. This fades to yellow on standing.

If the reaction be repeated and the solution acidified at once with acetic acid, a purple-red colour is produced.

*   Rothera suggests that the reaction be carried out by adding 3 drops of 5 per cent. sodium nitroprusside and 1-2 c.c. of ammonia to the dilute acetone and a small quantity of solid ammonium sulphate. A permanganate colour slowly develops, reaches a maximum in about 30 minutes and then fades away.

Creatinine does not react under these conditions; a brownish-red colour is given by aldehydes.

Salicylic Aldehyde Test.

1 gm. of solid potassium hydroxide is added to 10 c.c. of the acetone solution, and before it dissolves 10 drops of salicylic aldehyde are added. On warming to 70° a purple-red contact ring appears. If the potash has dissolved before adding the salicylic aldehyde the liquid becomes yellow, red, and finally purple-red.

Note.—The iodoform, nitroprusside and salicylic aldehyde reactions are carried out preferably in colourless solutions. The acetone should be separated by distillation and the distillate tested.

CHAPTER XI.

THE FATTY ACIDS.

THE fatty acids are the second products of oxidation of the primary alcohols, the aldehydes being the intermediate products. Secondary alcohols and ketones also give rise to fatty acids on oxidation, but the number of carbon atoms in the molecules of the fatty acids so formed is less than in the original secondary alcohol. Conversely, on reduction, fatty acids give aldehydes and primary alcohols, thus :—

$$CH_3 . CH_2OH \rightleftarrows CH_3 . CHO \rightleftarrows CH_3 . COOH.$$

The fatty acids are characterised by the presence of the carboxyl or —COOH group, and are sometimes called carboxylic acids. Their general formula is $C_nH_{2n}O_2$.
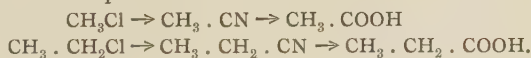
Constitution.

The fatty acids are monobasic acids : only one hydrogen atom is replaceable by metals, e.g. $C_2H_3O_2Na$.

One of the hydrogen atoms is attached to oxygen : phosphorus pentachloride replaces OH by Cl, giving acetyl chloride (p. 102), C_2H_3OCl. Hence $C_2H_3O . OH$. The sodium salt also yields acetyl chloride with phosphorus pentachloride, so that the acidic H is attached to an oxygen atom.
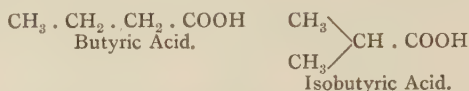
In the case of acetic acid, again, chlorine will directly substitute three hydrogen atoms, giving trichloracetic acid, $C_2Cl_3 . O_2H$, which is a monobasic acid containing OH replaceable by Cl with phosphorus chloride. Hence $C_2Cl_3 . OOH$. As trichloracetic acid yields chloroform on boiling with water whose constitution is certain, the three chlorine atoms have taken the place of three atoms attached to one carbon atom.

Hence $CCl_3 . C\underset{\diagdown OH}{\overset{\diagup O}{}}$ and $CH_3 . C\underset{\diagdown OH}{\overset{\diagup O}{}}.$
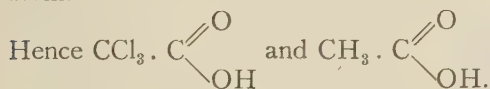
The formation of the acid from the alkyl halide, through the nitrile (p. 114) gives further proof :—

$$CH_3Cl \rightarrow CH_3 . CN \rightarrow CH_3 . COOH$$
$$CH_3 . CH_2Cl \rightarrow CH_3 . CH_2 . CN \rightarrow CH_3 . CH_2 . COOH.$$

Isomerism occurs amongst the higher members of the group, the first case appearing in the fourth member :—

$$CH_3 . CH_2 . CH_2 . COOH$$
Butyric Acid.

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \Big\rangle CH . COOH$$
Isobutyric Acid.

Fatty Acids occur widely distributed in nature, occasionally in the free state but more especially in combination with glycerol as the fats. Only acids containing an even number of carbon atoms occur in combination as fats, and as far as is known they all have a straight chain of carbon atoms. Several of the fatty acids are produced by fermentation by different micro-organisms.

Volatile and Non-Volatile Fatty Acids.

The lower members of the series of the fatty acids up to capric acid with 10 carbon atoms are volatile with steam and hence are termed the volatile fatty acids. They are separated in this way from the higher members which are not volatile with steam. They thus form two groups.

In the following list are given the names of the homologous series of hydrocarbons, primary alcohols, aldehydes and fatty acids :—

Number of Carbon Atoms.	Saturated Hydrocarbon.	Primary Alcohol — CH_2OH.	Aldehyde —CHO.	Fatty Acid —COOH.
1	Methane	Methyl	Formaldehyde	Formic
2	Ethane	Ethyl	Acetaldehyde	Acetic
3	Propane	Propyl	Propionic aldehyde	Propionic
4	Butane	Butyl	Butyric ,,	Butyric
5	Pentane	Amyl	Valeric ,,	Valerianic
6	Hexane	Hexyl	Caproic ,,	Caproic
7	Heptane	Heptyl	Oenanthic ,,	Oenanthic
8	Octane	Octyl	Caprylic ,,	Caprylic
9	Nonane	Nonyl	Pelargonic ,,	Pelargonic
10	Decane	Decyl	Capric ,,	Capric
11	Undecane	Undecyl	Undecylic ,,	Undecylic
12	Dodecane	Dodecyl	Lauric ,,	Lauric
13	Tridecane	—	Tridecylic ,,	Tridecylic
14	Tetradecane	Tetradecyl	Myristic ,,	Myristic
15	Pentadecane	—	—	Pentadecylic
16	Hexadecane	Cetyl	Palmitic ,,	Palmitic
17	Heptadecane	—	Margaric ,,	Margaric
18	Octadecane	Octadecyl	Stearic ,,	Stearic
19	Nonadecane	—	—	—
20	Eicosane	—	—	Arachic
21	Heneicosane	—	—	—
22	Docosane	—	—	Behenic
23	Tricosane	—	—	—
24	Tetracosane	—	—	Lignoceric
25	—	—	—	—
26	—	Ceryl	—	Cerotic
27	Heptacosane	—	—	—
28	—	—	—	—
29	—	—	—	—
30	—	Myricyl	—	Melissic

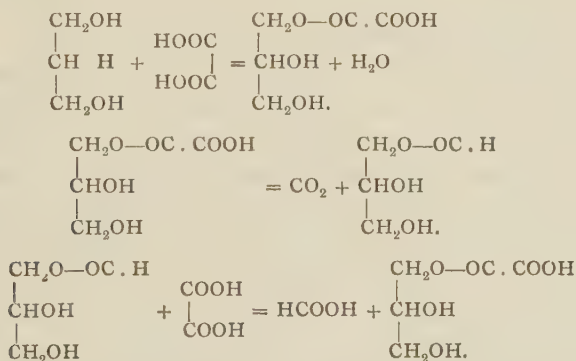## FORMIC ACID, H . COOH.

Preparation.

Formic acid was first prepared by distilling crushed ants with water —hence its name. The stings of some insects and plants also contain it, though the real poison is probably a much more complex substance. It occurs together with acetic and other lower fatty acids in urine. It can be obtained by oxidising methyl alcohol with potassium permanganate. It is formed in the decomposition of chloroform and chloral by alkali (p. 85), by the action of water upon hydrogen cyanide (p. 115).

Its alkaline salts are obtained by the combination of carbon monoxide with alkalies :—

$$CO + KOH = HCOOK.$$

This reaction takes place at 200°, or at a lower temperature under a pressure of 1-2 atmospheres.

It is most easily prepared by heating glycerol with oxalic acid. It has been shown by Chattaway that in this reaction glyceryl acid oxalate is formed; on raising the temperature, carbon dioxide is evolved and glyceryl monoformin is produced. On hydrolysing this ester with a further quantity of oxalic acid, formic acid is produced and the acid oxalate again formed. There is thus a continuous reaction :—

$$\begin{array}{l} CH_2OH \\ | \\ CH\ H\ + \\ | \\ CH_2OH \end{array} \begin{array}{l} HOOC \\ | \\ HOOC \end{array} = \begin{array}{l} CH_2O-OC . COOH \\ | \\ CHOH + H_2O \\ | \\ CH_2OH. \end{array}$$

$$\begin{array}{l} CH_2O-OC . COOH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} = CO_2 + \begin{array}{l} CH_2O-OC . H \\ | \\ CHOH \\ | \\ CH_2OH. \end{array}$$

$$\begin{array}{l} CH_2O-OC . H \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + \begin{array}{l} COOH \\ | \\ COOH \end{array} = HCOOH + \begin{array}{l} CH_2O-OC . COOH \\ | \\ CHOH \\ | \\ CH_2OH. \end{array}$$

Formic acid is produced from acetylene by dissolving it in fuming sulphuric acid, diluting with water and boiling with lime, or baryta. Acetaldehyde disulphonic acid is formed by addition. It decomposes, like chloral, on boiling with alkali (baryta) :—

$$\begin{array}{l} CH \\ ||| \\ CH \end{array} + 2HO . SO_2 . OH = \begin{array}{l} CH(SO_2 . OH)_2 \\ | \\ C \diagup^{O}_{\diagdown H} \end{array} + H_2O$$

$$\begin{array}{l} CH(SO_2 . OH)_2 \\ | \\ C \diagup^{O}_{\diagdown H} \end{array} + \begin{array}{l} H \\ | \\ OH \end{array} = CH_2 . (SO_2 . OH)_2 + HCOOH.$$
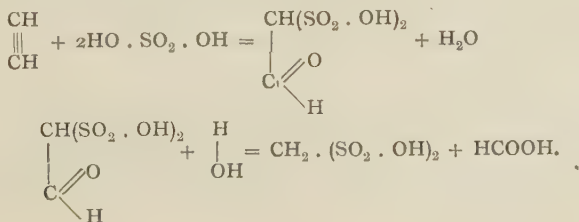
Barium methylene disulphonic acid is insoluble, whilst barium formate is soluble in water.

Formic acid is isolated from aqueous solutions by neutralising with alkali, evaporating and treating the dry salt with sulphuric acid, and distilling.

Properties.

Formic acid is a colourless volatile liquid with pungent odour. It has a sp. gr. of 1·221 at 20°, freezes at 8·3°, and boils at 100°. It is a very strong acid, about 12 times as strong as acetic acid, and produces blisters on the skin and intense irritation.

It dissolves in water, alcohol and ether, and in general properties resembles acetic acid.

The formates crystallise well and are prepared in the same way as acetates (p. 97). The lead and magnesium salts are insoluble in alcohol ; the corresponding acetates are soluble. The acids may therefore be separated by treating a concentrated solution of these salts with alcohol ; the formate is then precipitated. Potassium formate is almost insoluble in alcohol and may thus also be separated from the acetate, which is soluble.

Reactions and Detection.

A solution of formic acid must be exactly neutralised with soda, or ammonia, before the tests can be carried out. It is generally preferred to neutralise with ammonia and remove any excess by boiling. Solid formates are obtained by evaporating their solutions to dryness.

*   (1) On boiling a solution of a formate with dilute sulphuric acid, formic acid is evolved. Its pungent odour is only perceptible with strong solutions.

*   (2) On heating a solid formate with concentrated sulphuric acid, carbon monoxide is evolved and it may be ignited at the mouth of the test tube.

*   (3) Ethyl formate is formed when solid formates are heated with alcohol and concentrated sulphuric acid.

*   (4) A red solution containing ferric formate is obtained when ferric chloride, or ferric nitrate, is added to a *neutral* solution of a formate. On heating, a reddish-brown precipitate of basic ferric formate is produced.
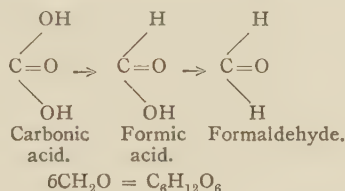
Formic acid differs from acetic acid in its reducing properties, which . are due to the presence of the aldehyde group CHO in its molecule.

*   (5) In concentrated solution it forms with silver nitrate a white

crystalline precipitate of silver formate. This precipitate darkens on standing owing to reduction to metallic silver. A precipitate is not formed in dilute solution, but the solution is reduced on heating with separation of metallic silver. The reduction is retarded in the presence of ammonia.

(6) On adding mercuric chloride solution and heating, a precipitate of mercurous chloride is produced, which, on further heating, may be reduced to metallic mercury.

Note.—It should be realised that formic acid is the first reduction product of carbonic acid : on further reduction, formaldehyde is formed. In nature aldol condensation leads to sugar.

$$
\begin{array}{ccc}
\mathrm{OH} & \mathrm{H} & \mathrm{H} \\
\diagup & \diagup & \diagup \\
\mathrm{C}{=}\mathrm{O} \;\rightarrow\; & \mathrm{C}{=}\mathrm{O} \;\rightarrow\; & \mathrm{C}{=}\mathrm{O} \\
\diagdown & \diagdown & \diagdown \\
\mathrm{OH} & \mathrm{OH} & \mathrm{H}
\end{array}
$$

Carbonic   Formic   Formaldehyde.
acid.   acid.

$$6CH_2O = C_6H_{12}O_6$$

ACETIC ACID, $CH_3 . COOH.$

Preparation.

Acetic acid is one of the few products made commercially by biological methods, i.e. by the oxidation of dilute alcohol by means of the micro-organism *Mycoderma aceti*, or "mother of vinegar". Pure dilute alcohol does not oxidise readily to acetic acid.

Red and white wine, cider, beer and malt, and sugar prepared from starch are the materials from which the vinegar is made. These materials contain in solution organic matter and various salts upon which the micro-organisms live and grow. Vinegar therefore contains, besides acetic acid, other organic acids, sugar, dextrin, and colouring matters which were present in the original material. The amount of acetic acid in the solution varies from about 3-12 per cent., the average quantity being about 5 per cent.

Vinegar is largely produced by the so-called quick vinegar process. By this means the alcoholic liquid is exposed to a large surface of air, so that acetification is as rapid as possible. Most commonly the alcoholic liquid is made to trickle slowly through wood shavings contained in a perforated barrel. The liquid, as it percolates, becomes acid. Usually the liquid is passed through a series so as to get complete conversion of the alcohol to acetic acid.

A large quantity of acetic acid is produced by the dry distillation

of wood, the crude material obtained in this way being termed pyro-ligneous acid (p. 59).   Tarry matter separates out on adding hydro-chloric acid to the solution which has been neutralised with lime and distilled to remove methyl alcohol and acetone.   The clear liquid is again neutralised and evaporated to dryness and the dry residue heated to decompose the empyreumatic products.   Comparatively pure acetic acid is obtained on distilling the residue with hydrochloric acid.   Pure acetic acid is prepared by distilling with potassium bichromate, or neutralising with soda and distilling the sodium salt, which has been heated to destroy tarry matter, with sulphuric, or hydrochloric, acid.

Preparation by the Oxidation of Alcohol with Permanganate.

14 gm. of potassium permanganate are dissolved in about 200 c.c. of water in a litre flask and 8 c.c. of concentrated sulphuric acid are added.   The flask is fitted with a reflux condenser and through the condenser a mixture of 5 c.c. of alcohol and 50 c.c. of water is slowly added.   The reaction must be kept moderate and, after all the alcohol has been added, the mixture is boiled for about 15 minutes.   The acetic acid is separated by distilling over about three-fourths of the liquid. The distillate will contain the acetic acid which may be tested for as described on p. 97.

In order to obtain pure acetic acid the distillate is neutralised and evaporated.   The dry salt is then distilled with sulphuric acid.   Acetic acid is volatile with steam and cannot be obtained by simple evaporation.

Properties.

Acetic acid is a colourless liquid with a characteristic pungent smell. The pure acid boils at 119° and distils without decomposition ; on cooling it crystallises in plates which melt at 17° and hence is termed glacial acetic acid ; its sp. gr. at 16·5° is 1·052.

The liquid is not inflammable, but its vapour burns with a blue flame.

It is miscible in all proportions with water, alcohol and ether. Heat is evolved on adding water to acetic acid and there is a con-traction in volume.

Acetic acid is a very corrosive liquid and dissolves oils, resins, camphor, gelatin and many metallic salts which are insoluble in water.   It is a very stable compound and is attacked only with difficulty by the most powerful oxidising agents.   It is not affected by nitric acid or chromic acid.   A solution of chromic acid in acetic acid is employed for oxidising hydrocarbons.   Chlorine converts it into chloracetic acids (p. 100).

As an acid, acetic acid forms salts. Most of the salts are soluble in water; the silver and mercurous salts are sparingly soluble; the sodium and potassium salts are soluble in alcohol. Some of the basic salts are insoluble.

The salts are prepared by boiling the acid with the oxide, or carbonate, of the metal until the solution is neutral, filtering and evaporating the solution until crystallisation begins. The metallic acetates, on being subjected to dry distillation, yield acetone.

Reactions and Detection.

Free acetic acid may be recognised by its odour. The acid solution is *exactly neutralised* with sodium hydroxide, or ammonia, and then tested. Neutral solutions of acetates may be tested directly. Insoluble (basic) acetates are converted into sodium acetate by boiling with sodium carbonate, filtering off the insoluble carbonate, neutralising and testing the filtrate :—

(1) On warming the solution with dilute sulphuric acid, the pungent odour of acetic acid is evolved.

(2) On adding ferric nitrate, or ferric chloride, the neutral solution gives a deep red liquid, which contains ferric acetate. An excess must be avoided. On boiling, the liquid becomes colourless and a brownish-red precipitate of basic ferric acetate is produced.

The cold red liquid is decolorised by adding dilute hydrochloric, or sulphuric, acid, but not by mercuric chloride solution.

(3) Concentrated solutions and dry acetates give the smell of ethyl acetate on heating with alcohol and concentrated sulphuric acid.

(4) On mixing a solid acetate with arsenious oxide and heating, cacodyl oxide, which has a garlic-like smell, is evolved. Only minute quantities should be used, as the product is *very poisonous :*—

$$4CH_3.COONa + As_2O_3 = (CH_3)_2As.O.As(CH_3)_2 + 2CO_2 + 2Na_2CO_3.$$

Propionic Acid, $CH_3.CH_2.COOH$.

Propionic acid is present with acetic acid in pyroligneous acid; it is found in sweat and is a product of putrefactive fermentation. It is most easily prepared by the oxidation of propyl alcohol with potassium bichromate and sulphuric acid.

Propionic acid closely resembles acetic acid in its properties: it is a liquid which boils at 140° and has a sp. gr. of ·996 at 19°. It mixes with water in all proportions, and may be separated from solution by adding calcium chloride, which causes it to float as an oily layer.

Butyric Acid, $CH_3.CH_2.CH_2.COOH$.

Butyric acid occurs in the free state in various animal and vegetable secretions and in the form of its glyceride—butyrin (see fats) ; butyrin is

always stated to exist in butter to the extent of about 6 per cent., but this compound could not be separated by Hurtley by the distillation of pure butter *in vacuo*. Since butyric acid results from the putrefaction of proteins and amino acids, it seems most probable that its occurrence in butter is due to the presence of butter milk which has not been removed and which has undergone decomposition. Its smell is always obvious in rancid butter.

Butyric acid is prepared by the butyric fermentation by butyric acid bacteria of glucose and other carbohydrates in the same way as lactic acid (p. 142). The filtered solution is evaporated, acidified and distilled. The acid distillate is neutralised, evaporated and the salt distilled with sulphuric acid.

Butyric acid is a colourless liquid with a pungent and disagreeable smell. Like propionic acid, it can be separated from aqueous solution by the addition of calcium chloride.

$$\textbf{Isobutyric Acid,} \quad \begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!\!>\!\!CH . COOH.$$
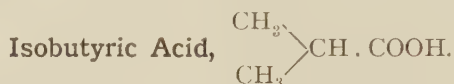
Isobutyric acid is found as the free acid, or as ester, in certain plants. It is a product of putrefaction of proteins and arises from the amino acid, valine.

It is very like normal butyric acid, but not so offensive in smell.

Valerianic, or Valeric, Acids, $C_4H_{10} . COOH.$

Four isomers are possible. The common valerianic acid is isovaleric acid, $\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!\!>\!\!CH . CH_2 . COOH$, which occurs in valerian root and various other animal and vegetable secretions. It is probably formed by the decomposition of leucine.

Methyl ethyl acetic acid, $\begin{matrix} CH_3 \\ C_2H_5 \end{matrix}\!\!\!>\!\!CH . COOH$, is also found in nature. This compound is optically active (see under lactic acid) and would be derived from isoleucine by putrefaction.

Normal valerianic acid, which has been found as a fermentation product, presumably of carbohydrate, most likely arises from amino acids in the same way as the other acids.

These acids are liquids with an unpleasant smell and behave in most respects like butyric acid.

Caproic to Myristic Acids.

The fatty acids with 6, 8 and 10 carbon atoms are found in combination in various fats. They are liquids slightly soluble in water.

The fatty acids with 12 and 14 atoms of carbon are solids of low melting-point, and are found as glycerides in small quantities in fats.

PALMITIC AND STEARIC ACIDS.

The principal higher fatty acids are palmitic and stearic acids with 16 and 18 atoms of carbon in their molecules respectively. These acids, together with oleic acid (p. 163), are obtained by the hydrolysis of fats. The liquid oleic acid is removed by pressure, and the

solid mixture of palmitic and stearic acids, "stearine," is used for making candles.

Palmitic acid was first made from palm oil.   Its best source is Japan wax, which consists almost entirely of the glyceride of palmitic acid.

Stearic acid is most conveniently made from Shea butter.   This material is composed almost entirely of the glycerides of oleic and stearic acids.

Stearic acid can be made from oleic acid (p. 163), which is an unsaturated compound, by reduction.

Properties.

The higher fatty acids are white odourless solids.   On heating, they melt at a low temperature, and on further heating they boil, giving off white vapours which condense on the cool parts of the test tube.

They are insoluble in water, slightly soluble in alcohol and readily soluble in ether.   The solubility in alcohol may be seen by adding some of the alcoholic solution to some alcohol containing a drop of dilute caustic soda and a drop of phenolphthalein.   The red colour of the latter is discharged.

They dissolve in dilute caustic alkali, aqueous or alcoholic, forming solutions of soap.

Soaps.

Soaps are the sodium and potassium salts of the higher fatty acids ; the former constitute hard soaps, the latter soft soaps.

(1) Solutions of soap in water have an alkaline reaction to litmus owing to partial hydrolysis of the salt.

(2) On adding excess of mineral acid (H_2SO_4) to a solution of soap in water, the fatty acids are liberated and form a precipitate which floats to the surface.

(3) On adding calcium chloride, or magnesium sulphate, to a solution of soap in water, a curdy precipitate of the calcium, or magnesium, salt is formed just as is obtained with hard water.

(4) On adding finely powdered sodium chloride to a soap solution, the soap is salted out as a curdy mass which clings to the sides of the vessel.

7 *

CHAPTER XII.

THE fatty acids behave like a saturated hydrocarbon towards the halogens, especially chlorine and bromine, substitution of hydrogen atoms in the chain of carbon atoms (not the COOH group) taking place. The most typical compounds are mono-, di- and tri-chloracetic acids.

In the case of the higher fatty acids containing three and more carbon atoms several isomers can be formed :—

$$CH_3 . CHBr . COOH \qquad CH_2Cl . CH_2 . COOH \qquad CH_2Cl . CH_2 . CH_2 . COOH$$
a-bromopropionic acid.   β-chloropropionic acid.   γ-chlorobutyric acid.
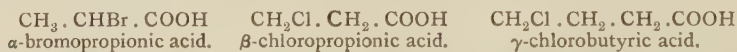
These acids are distinguished by using the Greek letters, that carbon atom next to the carboxyl group being called the a-carbon atom, the next β, the next γ, and so on.

Preparation.

The halogen-substituted fatty acids are prepared :—

(*a*) By the action of halogen upon the fatty acid in direct sunlight, or in the presence of iodine, or by the action of halogen upon the acid chloride (p. 102) which reacts more readily. Bromine preferably substitutes a hydrogen atom in the a-position, whilst chlorine does so in the β-position.

(*b*) By indirect methods such as :—

(1) addition of hydrogen halide to an unsaturated acid ;

(2) replacement of an OH group by means of phosphorus penta-chloride in hydroxy acids.

Monochloracetic Acid.

Chlorine is passed into boiling acetic acid, to which a little sulphur, or iodine, has been added :—

$$CH_3COOH + ICl_3 = CH_2Cl . COOH + ICl + HCl$$
$$ICl + Cl_2 = ICl_3.$$
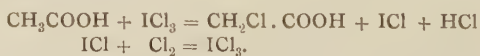
Monochloracetic acid is a colourless solid melting at 62° and boiling at 185-187°. It closely resembles acetic acid in its reactions.

Dichloracetic Acid.

Dichloracetic acid is usually prepared by heating chloral hydrate with potassium cyanide, or ferrocyanide :—

$$CCl_3 . CH(OH)_2 + KCN = CHCl_2 . COOH + HCN + KCl.$$

It is a liquid which boils at 190-191°.

Trichloracetic Acid.

Trichloracetic acid is prepared by oxidising chloral with concentrated nitric acid :—

$$CCl_3 . CHO + O = CCl_3 . COOH.$$
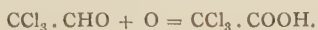
It is a colourless solid melting at 55° and boiling at 195°. On boiling with alkalies, it is converted into chloroform and carbonate :—

$$CCl_3 . COOH + NaOH = CHCl_3 + NaHCO_3.$$

It forms salt with bases and yields an acid chloride (p. 102) like acetic acid.

The acidity of these acids increases with the number of chlorine atoms ; trichloracetic acid is a strong acid almost equal to mineral acids.

CHAPTER XIII.

A. ACID, OR ACYL, CHLORIDES.

THE fatty acids, like the alcohols, contain a hydroxyl group. Phosphorus pentachloride and phosphorus trichloride act upon the acids forming the acid, or acyl, chloride :—

$$CH_3 . COOH + PCl_5 = CH_3 . CO . Cl + POCl_3 + HCl$$
$$3CH_3 . COOH + 2PCl_3 = 3CH_3 . CO . Cl + P_2O_3 + 3HCl.$$
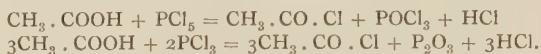
Preparation of Acetyl Chloride.

A distilling flask is fitted with a tap funnel and connected with a condenser and receiver. If the preparation be not carried out in a fume cupboard, the receiver should be connected with a tower containing soda lime to absorb hydrochloric acid.

25 gm. of glacial acetic acid are placed in the distilling flask and 20 gm. of phosphorus trichloride are slowly dropped upon it through the tap funnel. The flask is warmed upon a water-bath at 40-50° until the hydrochloric acid evolution has ceased ; the contents of the flask are then distilled from a water-bath. Acetyl chloride, which boils at 55°, passes over.

Properties of Acyl Chlorides.

Formyl chloride is not known. Acetyl chloride is a liquid with pungent smell ; other acyl chlorides are liquids, or solids. They fume in moist air and undergo decomposition into hydrochloric acid and the acid from which they are derived.

Reactions of Acyl Chlorides.

Acyl chlorides are rapidly decomposed by water, giving the acid and hydrochloric acid :—

$$CH_3COCl + H_2O = HCl + CH_3COOH.$$
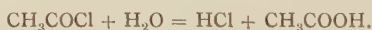
They react with alcohols giving esters (p. 66) :—

$$CH_3COCl + HOC_2H_5 = CH_3COOC_2H_5 + HCl.$$
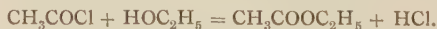
They react with ammonia giving amides (p. 105) :—

$$CH_3COCl + NH_3 = CH_3CONH_2 + HCl.$$
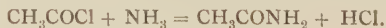
N.B.—The acyl chlorides, though decomposed by water, are sometimes only decomposed slowly and can be used in aqueous, or alkaline, solution for preparing esters, or for preparing acyl derivatives of amines. The process of introducing an acid radicle into the molecule of an alcohol,

or an amine, is known as acetylation or acylation, or arylation if aromatic acid chlorides be used. An alkaline solution of the alcohol, or of an amine, is shaken with the acyl chloride. The ester, or acyl derivative, is generally insoluble and can be filtered off and purified by crystallisation.

B. ACID ANHYDRIDES.

If acid chlorides be allowed to act upon the sodium salt of a fatty acid, an acid anhydride is formed :—

$$CH_3CO . Cl + NaOOC . CH_3 = NaCl + CH_3 . CO—O—OC . CH_3.$$
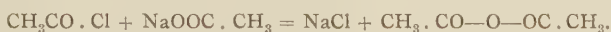
They can also be prepared by passing phosgene over the hot salts of the acids :—

$$2CH_3COONa + COCl_2 = (CH_3CO)_2 + CO_2 + 2NaCl.$$
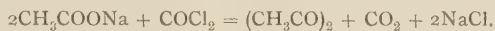
The constitution of these compounds is analogous to the ethers; two radicles are united by an oxygen atom. In the case of ethers, the two radicles are alkyl; in the case of anhydrides, they are acyl. They are sometimes called acyl oxides.

Mixed anhydrides can be prepared by using different acyl chlorides and different sodium salts of fatty acids :—

Ethers.   Anhydrides.
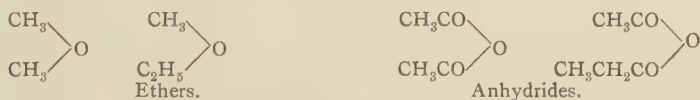
Preparation of Acetic Anhydride.

40 gm. of fused sodium acetate are placed in a retort which is connected to a condenser and receiver and fitted with a dropping funnel. 30 gm. of acetyl chloride are run in slowly and the contents of the flask are kept cold by immersion in cold water. The contents of the retort are well stirred and distilled. Acetic anhydride, which boils at 139°, passes over between 130° and 140°.

Properties.

The anhydrides are liquids possessing a pungent smell, but do not fume in the air.

Reactions.

The reactions of the anhydrides are the same as the acyl chlorides. They are decomposed by water giving the constituent acid :—

$$CH_3CO{\large>}O + H_2O = 2CH_3COOH.$$
$$CH_3CO$$
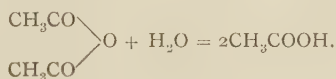
They yield esters with alcohols :—

$$CH_3CO{\large>}O + HOC_2H_5 = CH_3COOC_2H_5 + CH_3COOH.$$
$$CH_3CO$$
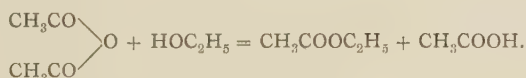
They yield amides with ammonia :—

$$CH_3CO{\diagdown\atop\diagup}\!\!\!> + NH_3 = CH_3CONH_2 + CH_3COOH.$$
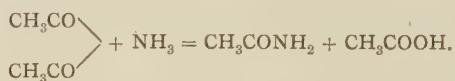
Like the acyl chlorides they are also used for acylating alcohols and compounds containing amino (NH_2) groups. The compound is boiled under an air condenser with the anhydride for some hours and poured into water. The acyl derivative is generally insoluble and is recrystallised from a suitable solvent.

THE amides are derivatives of fatty acids, in which the hydroxyl of the carboxyl group has been replaced by an amino (NH_2) group. They may also be regarded as derived from ammonia by the replacement of one of the hydrogen atoms by an acid radicle, e.g. :—

$$H . CO . NH_2 \qquad CH_3 . CO . NH_2$$
Formamide.   Acetamide.
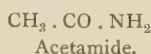
The amides resemble the primary amines in containing an amino (NH_2) group, but differ from them in most other respects.

Preparation.

Amides are prepared by several methods :—

(1) By the distillation of the ammonium salt of an acid :—

$$CH_3 . COONH_4 = H_2O + CH_3 . CO . NH_2.$$

(2) By the action of ammonia upon an acid chloride :—

$$CH_3 . COCl + NH_3 = CH_3 . CO . NH_2 + HCl.$$
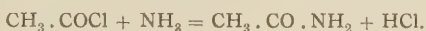
(3) By the action of ammonia upon an acid anhydride :—

$$\left. \begin{array}{c} CH_3 . CO \\ \\ CH_3 . CO \end{array} \right\rangle O + 2NH_3 = CH_3 . CO . NH_2 + CH_3 . COONH_4.$$
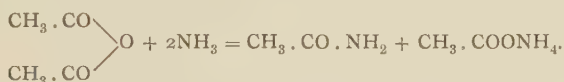
(4) By the action of ammonia upon an ester :—

$$CH_3 . COOC_2H_5 + NH_3 = CH_3 . CO . NH_2 + C_2H_5OH.$$

Properties.

The amides, except formamide which is liquid, are white crystalline solids generally easily soluble in water and alcohol. The substitution of a hydrogen atom in ammonia by acid radicles decreases its basic character; the amides are consequently neutral in reaction, but they are weak bases and form salts only with strong acids. They are unstable compounds and are readily decomposed by hydrolysis into their constituent acid and ammonia by boiling with water, acid or alkali, e.g.

$$CH_3 . CO . NH_2 + H_2O = CH_3 . COOH + NH_3.$$
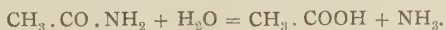
Acetamide.

Preparation.

. Acetamide is usually prepared by distilling ammonium acetate. About 50 gm. are melted in a basin and poured into a distilling flask attached to a wide air condenser. Water, acetic acid and ammonia first pass over on heating, but at about 180° acetamide commences to distil. It solidifies in the receiver. A better yield is obtained if, previous to the distillation, the ammonium acetate be heated in a sealed tube to 200° for 5 hours.

* Acetamide is more easily prepared by mixing together 5 c.c. of ethyl acetate and 5 c.c. of strong ammonia (sp. gr. ·880) in a small flask, closing the flask and allowing it to stand in a warm place for some time. The two layers gradually form a single homogeneous solution. The liquid is fractionally distilled : ammonia, alcohol and water pass over first and subsequently acetamide, which solidifies.

Properties and Reactions.

Acetamide is a white crystalline solid which melts at 82° and boils at 222°. It is easily soluble in water, alcohol and ether. It forms a salt with hydrochloric acid when the gas is passed into its solution in ether ; the salt is decomposed by water.

* (1) On boiling a solution of acetamide (about 50 c.c.) with an excess of caustic soda, ammonia is evolved, recognisable by its smell and action on red litmus paper. The boiling is continued until ammonia is no longer produced, and the solution is tested for acetic acid by carefully neutralising and adding ferric chloride (p. 97) :—

$$CH_3 . CO . NH_2 + NaOH = CH_3COONa + NH_3.$$

* (2) Acetamide is also hydrolysed by heating with acid in the same way :—

$$CH_3 . CO . NH_2 + HCl + H_2O = CH_3COOH + NH_4Cl.$$

The ammonia may be detected by adding excess of magnesium oxide and boiling ; the acid by neutralising and testing with ferric chloride.

* (3) On adding dilute hydrochloric acid and a few drops of sodium nitrite solution to a solution of acetamide, an effervescence of nitrogen occurs :—

$$CH_3 . CO . NH_2 + HNO_2 = CH_3 . COOH + H_2O + N_2.$$
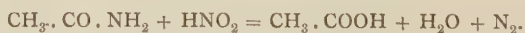
Acetic acid is formed and may be detected in the usual manner.

This reaction does not take place in the presence of acetic acid, only in presence of mineral acid. Amines and amino acids (see later) give this reaction in presence of acetic acid. The evolution of nitrogen under these conditions indicates the presence of an amino group (NH_2).

Amides on this account have probably the formula $CH_3 . C\underset{\displaystyle NH}{\overset{\displaystyle OH}{\diagdown}}$

in neutral solution, which changes in presence of mineral acids to

$CH_3 . C\overset{\displaystyle O}{\underset{\displaystyle NH_2}{\diagdown}}$. This other, or tautomeric formula, also explains the

existence of salts, e.g. $CH_3 . C\overset{\displaystyle O}{\underset{\displaystyle NH_2 . HCl}{\diagdown}}$ with strong acids, but not

with weak acids.

AMINES.

THE amines are compounds which are derived from ammonia by the replacement of one, two, or three of its hydrogen atoms by alkyl groups, e.g.—

CH_3NH_2 $\underset{C_2H_5}{\overset{CH_3}{>}}NH$ $(C_2H_5)_2 : NH$ $(CH_3)_3 \vdots N$

(Mono)methylamine.   Methyl-ethylamine.   Diethylamine.   Trimethylamine.
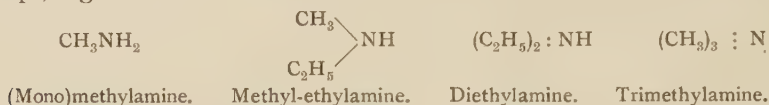
The three compounds are termed respectively a primary, a secondary, and a tertiary amine according as 1, 2, and 3 of the hydrogen atoms in ammonia are replaced by alkyl groups. If the alkyl groups are the same, they are known as simple amines, if different, as in methylethylamine, they are known as mixed amines.

Primary amines are characterised by the presence of the amino ($\cdot NH_2$) group; secondary amines are characterised by the presence of the imino ($:NH$) group; tertiary amines by $\vdots N$ completely substituted by alkyl groups.

Their relationship to the hydrocarbons is shown by their method of preparation from the alkyl halides. Primary amines may be regarded as derived from hydrocarbons in which a hydrogen atom has been replaced by the amino (NH_2) group, or as derived from alcohols in which the hydroxyl group has been replaced by the amino group.

Numerous amines occur in nature; they are products of decomposition of the amino acids, which lose carbon dioxide during putrefaction.

Preparation.

When an alkyl halide is treated with alcoholic ammonia, the halogen atom is replaced by the NH_2 group. This new compound again reacts with the alkyl halide, and the reaction continues until all the hydrogen atoms of ammonia are substituted by alkyl groups :—

$$CH_3Cl + NH_3 = HCl + CH_3 . NH_2$$
$$CH_3Cl + CH_3 . NH_2 = HCl + CH_3 . NH . CH_3$$
$$CH_3Cl + (CH_3)_2 : NH = HCl + (CH_3)_2 : N . CH_3$$
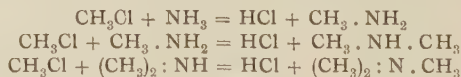
The tertiary amine combines with alkyl halide forming a quaternary ammonium salt :—

$$CH_3Cl + (CH_3)_3N = (CH_3)_3N\!\!\begin{array}{c} \diagup CH_3 \\ \diagdown Cl. \end{array}$$
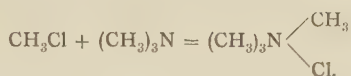
The three amines are strong bases and form salts with the hydrochloric acid.

A mixture of the four compounds is obtained.

Separation of the Amines.

The separation of the mixture of amines is not easily effected. Except the quaternary compound, the amines are liberated from their salts (see below) by caustic alkali and removed by distillation. The quaternary compound remains in the distilling flask. The easiest method of obtaining the products, though it involves the loss of the primary amine, is by the action of nitrous acid (see below). Nitrous acid converts the primary amine into the corresponding alcohol. The secondary amine is converted into a nitroso-amine, which is a yellowish oil insoluble in water. The tertiary amine is not affected. The solution, after filtration, is therefore made alkaline and distilled. The tertiary amine passes into the distillate. The nitroso-amine is converted to the amine by boiling with hydrochloric acid : on then making alkaline, it is obtained by distillation. The loss of the primary amine is of no great consequence as it can be prepared by several other methods.

Primary amines can also be prepared :—

(1) By the hydrolysis of isocyanates :—

$$CH_3 . CH_2 . N . CO + 2NaOH = CH_3 . CH_2 . NH_2 + Na_2CO_3.$$
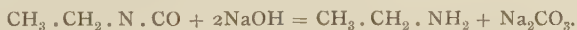
Primary amines were first prepared by this reaction by Wurtz in 1849 ;

(2) by the reduction of nitriles (p. 120) :—

$$CH_3 . CN + 2H_2 = CH_3 . CH_2 . NH_2 ;$$

(3) by the reduction of oximes :—

$$CH_3 . CH : NOH + 2H_2 = CH_3 . CH_2 . NH_2 + H_2O ;$$

(4) by the action of bromine on an amide :—

$$CH_3 . CO . NH_2 + Br_2 = CH_3 . CO . NHBr + HBr,$$
<div align="center">Acetobromamide</div>

and the decomposition of the bromamide by heating with excess of sodium hydroxide. Hydrobromic acid is removed and an isocyanate is formed :—

$$CH_3 . CO . NHBr + NaOH = CH_3 . N . CO + NaBr + H_2O$$
<div align="center">Methyl
isocyanate.</div>

the isocyanate on hydrolysis gives the primary amine :—

$$CH_3 . N . CO + 2NaOH = CH_3 . NH_2 + Na_2CO_3.$$

These reactions involving the preparation of a primary amine, and its decomposition by nitrous acid, serve for passing from a lower series of compounds to a higher series, and *vice versa*, thus methyl alcohol to ethyl alcohol :—

$$CH_3OH \rightarrow CH_3I \rightarrow CH_3.CN \rightarrow CH_3.CH_2.NH_2 \rightarrow CH_3.CH_2OH$$

ethyl alcohol to methyl alcohol :—

$$CH_3.CH_2OH \rightarrow CH_3.COOH \rightarrow CH_3.CONH_2 \rightarrow CH_3.NCO \rightarrow CH_3NH_2 \rightarrow CH_3OH.$$

Properties.

The lower members, whether primary, secondary, or tertiary, are gases, the next members are liquids, the highest members are solids. They have a characteristic and peculiar smell, which is pungent like ammonia and "fishy" in the lower members.   The lower members are easily soluble in water and closely resemble ammonia.

Like ammonia, they are strong bases which turn red litmus blue and unite with acids to form salts :—

$$CH_3.NH_2 + HCl = CH_3.NH_2{\Large\langle}{}^{H}_{Cl}$$

$$(CH_3)_3N + HCl = (CH_3)_3 \equiv N{\Large\langle}{}^{H}_{Cl}$$

These salts are frequently deliquescent, and generally soluble in water, sometimes in alcohol, chloroform, and other organic solvents. The bases are liberated on adding sodium hydroxide to their solution.

The salts yield double salts with gold, platinum chloride, etc. :—

$$CH_3.NH_2HCl + AuCl_3 = CH_3.NH_2.HCl.AuCl_3$$
$$2CH_3.NH_2HCl + PtCl_4 = (CH_3NH_2HCl)_2.PtCl_4.$$

These are of use for determining the molecular weight of the base (p. 34).

Reactions of a Primary Amine (Methylamine).

(1) On treating a solution of methylamine hydrochloride, or about ·2 gm. of the solid, with sodium hydroxide, the base is liberated :—

$$CH_3.NH_2.HCl + NaOH = CH_3.NH_2 + NaCl + H_2O.$$

Methylamine resembles ammonia in odour, but is "fishy," turns red litmus blue, and fumes with vapours of hydrochloric acid.   It differs from ammonia in being combustible.

(2) A double salt is formed on adding an alcoholic solution of gold, or platinum, chloride to an alcoholic solution of methylamine hydrochloride.

(3) *Action of Nitrous Acid.*—On adding dilute acetic acid and a few drops of sodium nitrite solution to a solution of methylamine

hydrochloride, there is an evolution of nitrogen (the gas extinguishes a glowing splinter) and methyl alcohol is formed :—

$$CH_3 . NH_2 . HCl + NaNO_2 = CH_3OH + N_2 + NaCl + H_2O.$$

This reaction is characteristic of primary amines; they are converted into the corresponding alcohol.

(4) *Carbylamine Reaction.*—On warming a solution of methylamine hydrochloride with *one drop* of chloroform and alcoholic sodium hydroxide, methylcarbylamine, or isocyanide, is formed :—

$$CH_3 . NH_2 + CHCl_3 + 3NaOH = CH_3 . N{\equiv}C + 3NaCl + 3H_2O.$$
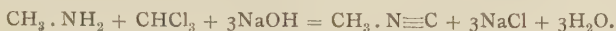
(5) *Reaction with Acetyl Chloride.*—Primary amines react with acetyl chloride, benzoyl chloride, etc., to form substituted amides :—

$$CH_3 . NH_2 + CH_3COCl = HCl + CH_3 . CO{-}NH . CH_3.$$

These compounds are crystalline and serve for identifying the amine.

Reactions of a Secondary Amine (Dimethylamine).

(1) Dimethylamine is liberated from a solution of dimethylamine hydrochloride on treatment with sodium hydroxide :—

$$(CH_3)_2 : NH . HCl + NaOH = (CH_3)_2 : NH + NaCl + H_2O.$$

It is a gas with an ammoniacal and fishy odour and resembles methylamine.

(2) A double salt is formed with alcoholic solutions of the heavy metals.

(3) *Action of Nitrous Acid.*—On adding dilute acetic acid and some nitrite to a solution of dimethylamine hydrochloride, there is no evolution of nitrogen, but dimethylnitrosamine is formed :—

$$(CH_3)_2 : NH . HCl + NaNO_2 = (CH_3)_2 : N . NO + NaCl + H_2O.$$
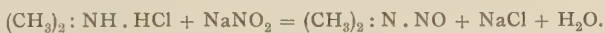
Dimethylnitrosamine is a yellowish oil which is volatile with steam and can thus be separated.

It is reconverted into the secondary amine by the action of concentrated hydrochloric acid :—

$$(CH_3)_2 : N . NO + 2HCl = (CH_3)_2 : NH, HCl + NOCl.$$
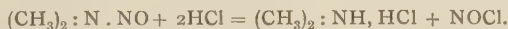
(4) *Carbylamine Reaction.*—Dimethylamine and secondary amines do not yield isocyanides with chloroform and alcoholic potash.

(5) *Reaction with Acetyl Chloride.*—Secondary amines form with acetyl chloride, etc., crystalline substituted amides which are useful for purposes of identification :—

$$CH_3COCl + HN(CH_3)_2 = HCl + CH_3CO{-}N(CH_3)_2.$$

Reactions of a Tertiary Amine (Trimethylamine).

* (1) Trimethylamine is evolved on adding excess of alkali to a solution of its hydrochloride :—

$$(CH_3)_3 : N : HCl + NaOH = (CH_3)_3 : N + NaCl + H_2O.$$
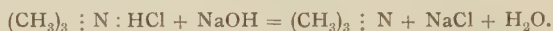
It is a gas like the primary and secondary amines with a fishy ammoniacal odour and is combustible.

(2) Double salts are formed on adding alcoholic solutions of gold chloride, etc., to an alcoholic solution of trimethylamine hydrochloride.

* (3) *Action of Nitrous Acid.*—Trimethylamine does not react with nitrous acid.

* (4) *Carbylamine Reaction.*—This reaction is not given by trimethylamine and tertiary amines.

(5) *Reaction with Acetyl Chloride.*—Tertiary amines do not react.

Quaternary Ammonium Salts.—Tertiary amines combine with alkyl iodides to form a quaternary ammonium salt :—

$$(CH_3)_3 : N + CH_3I = (CH_3)_3 : N {\overset{\displaystyle CH_3}{\underset{\displaystyle I.}{<}}}$$
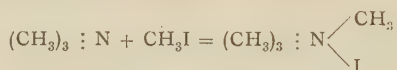
In this compound the halogen atom can be replaced by the hydroxyl group :—

$$(CH_3)_3 : N {\overset{\displaystyle CH_3}{\underset{\displaystyle I}{<}}} + AgOH = AgI + (CH_3)_3 : N {\overset{\displaystyle CH_3}{\underset{\displaystyle OH.}{<}}}$$

The hydroxide is a strong base, approaching the strength of the alkaline hydroxides.

The quaternary ammonium compounds are decomposed on heating :—

$$(CH_3)_3 : N {\overset{\displaystyle CH_3}{\underset{\displaystyle I}{<}}} = (CH_3)_3 : N + CH_3I$$

$$(CH_3)_3 : N {\overset{\displaystyle CH_3}{\underset{\displaystyle OH}{<}}} = (CH_3)_3 : N + CH_3OH.$$
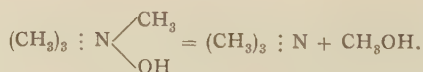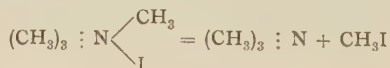
CHAPTER XVI.

CYANOGEN COMPOUNDS.

$$\text{(1) } \textbf{Cyanogen, } \begin{matrix} CN \\ | \\ CN. \end{matrix}$$

Cyanogen, as it contains only the two elements carbon and nitrogen, is the simplest carbon compound containing nitrogen. It is present in the gases of the blast furnace and is formed on passing electric sparks between carbon poles in an atmosphere of nitrogen, and on treating ammonium oxalate, or oxamide (p. 140), with dehydrating agents, e.g. phosphorus pentoxide :—

$$\begin{matrix} COONH_4 \\ | \\ COONH_4 \end{matrix} \rightarrow \begin{matrix} CONH_2 \\ | \\ CONH_2 \end{matrix} = 2H_2O + \begin{matrix} CN \\ | \\ CN. \end{matrix}$$
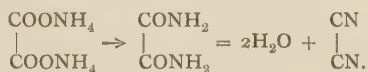
Preparation.

Cyanogen is most readily prepared by heating mercuric cyanide :—

$$Hg(CN)_2 = \begin{matrix} CN \\ | \\ CN \end{matrix} + Hg.$$
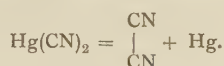
On heating a small quantity of mercuric cyanide in a dry test tube, white fumes are given off which condense on the cooler parts of the tube. On igniting the gas at the open end, it will be observed to burn with its characteristic pink flame.

Cyanogen is also prepared by heating a concentrated solution of 1 part of potassium cyanide with 2 parts of copper sulphate dissolved in 4 parts of water. A yellow precipitate of cupric cyanide, $Cu(CN)_2$, is first formed and this decomposes into cyanogen and cuprous cyanide, CuCN :—

$$4KCN + 2CuSO_4 = 2Cu(CN) + (CN)_2 + 2K_2SO_4.$$
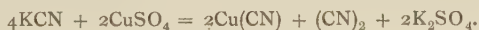
Properties.

Cyanogen is a colourless gas with a peculiar pungent smell and has been condensed to a liquid. It burns with a pink flame forming carbon dioxide and nitrogen. It is easily soluble in water and alcohol and is intensely poisonous.

Reactions.

Cyanogen and the other compounds of this group resemble the halogens very closely in their properties, thus, on passing cyanogen into alkali, it is converted into alkali cyanide and cyanate :—

$$(CN)_2 + 2KOH = KCN + KOCN + H_2O$$
$$Cl_2 + 2KOH = KCl + KOCl + H_2O.$$

Cyanogen is converted by hydrolysis, on boiling with acids, into oxalic acid and ammonia :—

$$\begin{matrix} CN \\ | \\ CN \end{matrix} + 4H_2O = \begin{matrix} COOH \\ | \\ COOH \end{matrix} + 2NH_3.$$
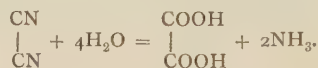
The compounds of this group which contain the CN radicle are termed nitriles, because on hydrolysis with acids they are converted into the corresponding acid. Cyanogen is the nitrile of oxalic acid, or oxalonitrile.

(2) Hydrogen Cyanide, or Prussic Acid, HCN.

Hydrogen cyanide is present in numerous plants, e.g. in laurel leaves, bitter almonds, cherry and peach kernels, usually in combination with glucose and benzaldehyde as the glucoside amygdalin (p. 282), which is hydrolysed by acids, or by enzymes in the plant, into its constituents. It is formed by the oxidation of glycine and other amino acids ; it may thus be produced in animals and plants : the latter probably then combine it with glucose to form a glucoside, the former apparently combine it with sulphur to form sulphocyanide, or thiocyanate, which is present in saliva and other secretions.

Preparation.

A dilute solution of hydrogen cyanide is obtained by distilling potassium ferrocyanide, or a cyanide, with dilute sulphuric acid :—

$$2K_4Fe(CN)_6 + 3H_2SO_4 = 6HCN + K_2FeFe(CN)_6 + 3K_2SO_4$$
$$\text{Potassium}$$
$$\text{ferrous ferrocyanide.}$$
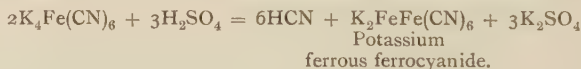
A small flask is connected with a condenser by means of a bent tube ; the open end of the condenser is dipped into water in a test tube containing a drop of strong caustic soda. 10 c.c. of a cold saturated solution of potassium ferrocyanide and 20 c.c. of 20 per cent. sulphuric acid are put in the flask. On heating, hydrogen cyanide distils over and is converted into sodium cyanide. Its presence is tested for by converting it into Prussian blue by heating in alkaline solution with a ferrous salt, acidifying, and adding a drop of ferric chloride. The distillation should be carried out in the fume cupboard.

Note.—Carbon monoxide is obtained when anhydrous potassium ferro-cyanide is heated with concentrated sulphuric acid.

Hydrogen cyanide is also obtained by dehydrating ammonium formate, or formamide, with phosphorus pentoxide.

Pure anhydrous hydrogen cyanide is prepared by distilling potassium cyanide with moderately concentrated sulphuric acid, passing the gas over anhydrous calcium chloride and collecting the distillate in a receiver cooled by ice.

Properties.

Pure hydrogen cyanide is a colourless, mobile liquid having a specific gravity of ·697 at 18°. It becomes a crystalline solid at − 15° and it boils at 26·5°. It has a peculiar smell, resembling that of oil of bitter almonds and is intensely poisonous. It burns with a violet flame and is easily soluble in water and alcohol.

It is a very weak acid and turns blue litmus only a faint red.

Reactions.

Aqueous solutions of hydrogen cyanide are unstable and slowly undergo decomposition into ammonium formate :—

$$HCN + 2H_2O = HCOONH_4.$$
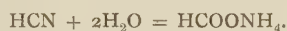
The pure acid is also rapidly decomposed by concentrated hydrochloric acid. Formamide is first formed and this passes into formic acid and ammonium chloride :—

$$HCN + H_2O = HCONH_2$$
$$HCONH_2 + HCl + H_2O = HCOOH + NH_4Cl.$$
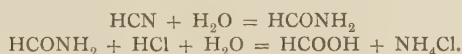
Hydrogen cyanide is thus the nitrile of formic acid.

The salts of hydrocyanic acid are decomposed in the same way on boiling their aqueous solutions :—

If about 20 c.c. of a 1 per cent. potassium cyanide solution be boiled for some time it is converted into ammonia and potassium formate. The ammonia is readily detected by its action on red litmus paper and the formate may be detected by testing the solution, after the ammonia has been given off, with (1) ferric chloride and with (2) mercuric chloride, as on p. 95.

Hydrogen cyanide in alcoholic solution is reduced by sodium to methylamine :—

$$HCN + 2H_2 = CH_3 . NH_2.$$
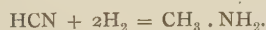
Metallic Cyanides.

Hydrogen cyanide resembles hydrochloric acid in behaviour, forming salts with alkalies and metallic hydroxides. The alkaline salts

crystallise like sodium and potassium chloride; the silver salt is white and insoluble in water and acids, but soluble in ammonia. Silver cyanide, unlike silver chloride, is decomposed by boiling with mineral acids forming hydrogen cyanide.

The chief salt is potassium cyanide which is used extensively for extracting gold and in electroplating.

Preparation of Potassium Cyanide.

Potassium cyanide is prepared by fusing potassium ferrocyanide :—

$$K_4Fe(CN)_6 = 4KCN + N_2 + FeC_2.$$

* On heating about 1 gm. of potassium ferrocyanide in a crucible to redness, allowing to cool, extracting the mass with water and filtering, the solution will be found to contain potassium cyanide as shown by the test on p. 117.

In this reaction the whole of the nitrogen of the ferrocyanide is not obtained as cyanide; if potassium ferrocyanide be fused with potassium carbonate, a mixture of cyanate and cyanide is formed :—

$$K_4Fe(CN)_6 + K_2CO_3 = 5KCN + KOCN + CO_2 + Fe.$$

If potassium ferrocyanide be fused with sodium, a mixture of potassium and sodium cyanides results :—

$$K_4Fe(CN)_6 + 2Na = 4KCN + 2NaCN + Fe.$$

The cyanides dissolve in water leaving the iron and are obtained by evaporation.

Large quantities of cyanide are now prepared by two other methods :—

(1) By heating sodium with charcoal in a current of ammonia at 400°. Sodamide is formed and converted into sodium cyanamide (p. 133) :—

$$2NH_3 + Na_2 = 2NaNH_2 + H_2$$
$$2NaNH_2 + C = Na_2CN_2 + 2H_2.$$
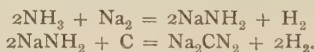
On raising the temperature to 800°, the sodium cyanamide and charcoal react, forming sodium cyanide :—

$$Na_2CN_2 + C = 2NaCN.$$

Sodium cyanide is formed directly at 800° :—

$$NaNH_2 + C = NaCN + H_2.$$

(2) By heating beet-sugar molasses to 1000°. At this temperature the trimethylamine is decomposed into hydrogen cyanide and methane :—

$$(CH_3)_3N = HCN + 2CH_4.$$

Sodium cyanide is prepared by passing the gases into sodium hydrate and evaporating the solution.

Metallic gold dissolves in potassium cyanide solution in the presence of air, or other oxidising agent :—

$$2Au + 4KCN + H_2O + O = 2KAu(CN)_2 + 2KOH,$$

· forming a double cyanide from which the gold is obtained by electrolysis.

Double Cyanides.

The alkali cyanides dissolve the insoluble cyanides of silver, gold, and other heavy metals forming the double cyanides :—

$$KCN + AgCN = KAg(CN)_2.$$

On adding a few drops of a 1 per cent. solution of potassium cyanide to a few drops of silver nitrate solution, a white precipitate of silver cyanide is formed :—

$$KCN + AgNO_3 = AgCN + KNO_3.$$

On adding more potassium cyanide solution, the precipitate dissolves forming the double salt. The double salt is decomposed with the formation of silver cyanide by adding dilute nitric acid :—

$$KAg(CN)_2 + HNO_3 = KNO_3 + HCN + AgCN.$$

The double cyanides are extensively used in electroplating; on electrolysis the compound is decomposed with the formation of potassium and $Ag(CN)_2$ ions at the cathode and anode respectively. The double cyanide is reduced at the cathode, the silver being deposited. Silver is used as the anode and is dissolved by the $Ag(CN)_2$ ions forming $2AgCN$, which is soluble in potassium cyanide giving the double cyanide. The reactions are thus :—

$$KAg(CN)_2 \rightarrow K + Ag(CN)_2 \qquad Ag + Ag(CN)_2 \rightarrow 2AgCN$$
$$K + KAg(CN)_2 \rightarrow 2KCN + Ag \qquad AgCN + KCN \rightarrow KAg(CN)_2.$$
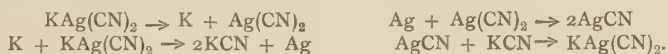
Tests for Cyanides.

(1) The smell of hydrogen cyanide, either before or after acidifying the solution with dilute nitric acid and warming, is an indication of the presence of a cyanide.

(2) The formation of silver cyanide, by adding silver nitrate to a solution acidified with nitric acid, or better by holding a drop of silver nitrate on a glass rod in the vapour of the solution in (1), also indicates the presence of a cyanide.

(3) The formation of Prussian blue by boiling the solution with a ferrous salt and alkali, acidifying and then adding a drop of ferric chloride is characteristic.

(4) The formation of ferric thiocyanate by adding to the solution a drop of ammonium sulphide, evaporating to dryness, acidifying with hydrochloric acid and adding a drop of ferric chloride, is the most delicate way of detecting a cyanide.

If a cyanide be present with other organic substances, e.g. in stomach contents, etc., in cases of poisoning, the material is acidified

with a non-volative organic acid, such as tartaric acid, and distilled. The distillate is tested for hydrogen cyanide as above.

Complex Cyanides.

The cyanides of sodium and potassium are converted into ferrocyanides on boiling their solutions with ferrous salts in alkaline solution. This reaction is made use of in testing for nitrogen in organic compounds.

Potassium Ferrocyanide, $K_4Fe(CN)_6$.

Preparation.

Potassium ferrocyanide is prepared by fusing together protein residues, such as blood, or horn, or leather, with scrap iron and potassium carbonate. The fused mass is extracted with water and the yellow solution which results is evaporated down until it crystallises.

Potassium ferrocyanide is also prepared from the hydrogen cyanide, which is a by-product in the manufacture of coal gas. The hydrogen cyanide is absorbed by iron oxide in the "purifiers". By boiling this material with lime, calcium ferrocyanide is formed; potassium ferrocyanide is obtained by treating it with potassium carbonate. Sometimes the hydrogen cyanide is converted into sodium ferrocyanide by passing it into an alkaline solution of ferrous salts.

Properties and Reactions.

Potassium ferrocyanide forms large yellow crystals (yellow prussiate of potash) which are easily soluble in water.

* (1) On adding concentrated hydrochloric acid to a saturated solution of potassium ferrocyanide, hydroferrocyanic acid is thrown down as a white precipitate. It turns blue on filtering owing to decomposition and oxidation.

* (2) On adding a drop of ferric chloride solution to a solution of potassium ferrocyanide, a blue precipitate (Prussian blue) is formed :—

$$3K_4Fe(CN)_6 + 4FeCl_3 = Fe_4[Fe(CN)_6]_3 + 12KCl.$$

(3) Other metals also form insoluble ferrocyanides if their solutions be added to a solution of potassium ferrocyanide.

Zinc ferrocyanide is white, copper ferrocyanide is reddish-brown, uranium ferrocyanide is brown.

Sodium Nitroprusside, $Na_2Fe(CN)_5 . NO$.

Potassium ferrocyanide is converted into nitroprusside by the action of moderately concentrated nitric acid; potassium nitrate crystallises out and is removed. The solution, on neutralisation with sodium carbonate, yields sodium nitroprusside on evaporation.

Sodium nitroprusside, $Na_2Fe(CN)_5 . NO + 2H_2O$, forms beautiful red rhombic prisms which are easily soluble in water; the solution is a sensitive reagent for sulphides, acetone, etc.

Potassium Ferricyanide, $K_3Fe(CN)_6$.

Preparation.

Potassium ferricyanide is formed by the oxidation of potassium ferrocyanide by means of chlorine, or bromine :—

On adding a slight excess of bromine water to some potassium ferrocyanide solution and boiling off the excess of bromine, the colour changes from yellow to brown-red, and on evaporation of the solution red crystals of potassium ferricyanide are obtained.

Properties and Reactions.

Potassium ferricyanide, or red prussiate of potash, is a red crystalline substance soluble in water, giving a reddish-yellow solution.

(1) On adding ferric chloride to its solution it turns dark brown.

(2) On adding a solution of a ferrous salt to its solution, a deep-blue precipitate (Turnbull's blue) is formed :—

$$2K_3Fe(CN)_6 + 3FeSO_4 = Fe_3[Fe(CN)_6]_2 + 3K_2SO_4.$$
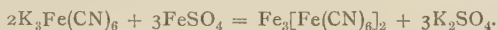
(3) In alkaline solution it is decomposed into potassium ferrocyanide and oxygen; it acts therefore as an oxidising agent :—

$$2K_3Fe(CN)_6 + 2KOH = 2K_4Fe(CN)_6 + H_2O + O.$$
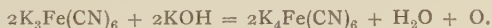
Thus, if some litharge be added to a solution of potassium ferri-cyanide rendered alkaline with sodium hydroxide and warmed, it becomes brown owing to the formation of lead peroxide, PbO_2. The solution may be tested for ferrocyanide by filtering, acidifying, and adding ferric chloride which gives a precipitate of Prussian blue.

Alkyl Cyanides—Nitriles.

Preparation.

When potassium cyanide is treated with an alkyl iodide, or alkyl sulphuric acid, the alkyl cyanide is obtained :—

$$KCN + CH_3I = KI + CH_3CN$$
$$KCN + C_2H_5OSO_2OK = C_2H_5CN + K_2SO_4.$$

These compounds are also formed by treating the amides of the corresponding acid with phosphorus pentoxide :—

$$CH_3CONH_2 = H_2O + CH_3CN.$$
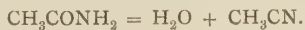
Properties and Reactions.

The lower members of the series are liquids with peculiar smell, and are more or less soluble in water; the higher members are solids.

Like hydrogen cyanide, they are hydrolysed by acids, or alkalies, into the corresponding acid :—

$$CH_3CN + 2H_2O = CH_3COOH + NH_3.$$
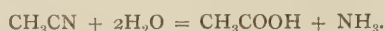
If hydrolysed by water, the acid amide is formed :—

$$CH_3CN + H_2O = CH_3CONH_2.$$
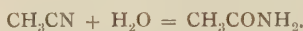
On reduction, they are converted into primary amines :—

$$CH_3CN + 2H_2 = CH_3 . CH_2 . NH_2.$$
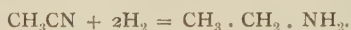
Alkyl Isocyanides—Isonitriles.

If silver cyanide be treated with alkyl iodides, isocyanides, compounds isomeric with the above, are formed :—

$$CH_3I + AgCN = CH_3NC + AgI.$$

It would thus appear that silver cyanide has a different structure to potassium cyanide, or that in the reaction a rearrangement occurs :—

$$KCN \rightarrow KNC \rightarrow AgNC.$$

These compounds are also formed by heating primary amines (p. 111) with chloroform and potash :—

$$CH_3NH_2 + CHCl_3 + 3KOH = CH_3NC + 3KCl + 3H_2O.$$
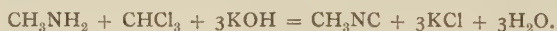
They are liquids with an abominable smell : on hydrolysis they give the corresponding amine and formic acid :—

$$CH_3NC + 2H_2O = CH_3NH_2 + HCOOH.$$

CHAPTER XVII.

Cyanic Acid, HOCN, or HNCO.

Cyanic acid is formed by distilling cyanuric acid, $C_3H_3O_3N_3$.

A small quantity of cyanuric acid is placed in a small bulb blown upon the end of a glass tube and the glass tubing is bent at an angle. The end of the glass tube leads into a test tube surrounded by a freezing mixture. On heating, the cyanuric acid is decomposed and cyanic acid collects in the test tube as a liquid.

Cyanic acid is only stable below $0°$ and is a mobile, volatile liquid with a strong acid reaction and with a smell resembling that of glacial acetic acid. It produces blisters upon the skin.

Pure cyanic acid, on exposure to the air, polymerises to cyamelide and cyanuric acid, with explosiveness; a small quantity, such as prepared above, polymerises with a cracking noise.

It is an extremely unstable substance; its aqueous solution above $0°$ decomposes giving carbon dioxide and ammonia :—

$$HOCN + H_2O = CO_2 + NH_3.$$
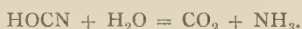
It is the nitrile of carbonic acid.

Its salts are more stable than the free acid.

Potassium Cyanate, KOCN.

Potassium cyanate is formed by the oxidation of potassium cyanide by a variety of oxidising agents, air, lead oxide, potassium permanganate, or sodium hypochlorite.

It may be prepared from potassium cyanide as follows :—

About 1 gm. of potassium cyanide is heated in a crucible in a fume cupboard until it melts. Lead oxide is added in small quantities to the fused mass so long as visible reduction occurs. The mass, when cold, is extracted with water; potassium cyanate crystallises out on evaporation.

It may also be conveniently prepared from potassium ferrocyanide: a mixture of 4 parts of potassium ferrocyanide and 3 parts of potassium bichromate are carefully heated in an iron dish, avoiding the formation of ammonia. The potassium cyanate is extracted with water.

Potassium cyanate forms shining leaflets, or quadratic plates. It dissolves readily in cold water, and is insoluble in absolute alcohol.

In aqueous solution, it is unstable and decomposes forming ammonium and potassium carbonates :—

$$2KOCN + 4H_2O = K_2CO_3 + (NH_4)_2CO_3.$$

This decomposition can be seen with the solution prepared above ; carbon dioxide is evolved on adding sulphuric acid, and the presence of ammonia may be shown by making alkaline, warming, and testing with red litmus.

Ammonium Cyanate.

Ammonium cyanate is prepared by bringing cyanic acid into contact with dry ammonia.

It is a white crystalline powder soluble in water ; the aqueous solution on evaporation yields urea (below).

The salts of cyanic acid with the heavy metals are insoluble and are formed from potassium cyanate by double decomposition. Silver cyanate is a white precipitate decomposed by dilute nitric acid.

UREA.

Urea is the chief nitrogenous constituent of the urine in which it is present to extent of about 2 per cent. It was first isolated from evaporated urine in 1773 by Rouelle by extraction with alcohol. Later it was prepared from concentrated urine as nitrate (see below). On analysis it was found to have the formula CH_4ON_2, and on decomposition gave carbon dioxide and ammonia.

Preparation.

(I) From urine.

(i) About 25 or 50 c.c. of urine are evaporated to dryness on the water-bath. The dry residue is treated with about 10 c.c. of acetone allowing the solvent to boil on a hot water-bath. The acetone is poured off into a clean vessel and allowed to evaporate (not in the neighbourhood of a flame). Urea crystallises out in long silky needles and is recrystallised from alcohol. A yield of about 1 gm. per 50 c.c. of urine should be obtained.

(ii) 100 c.c. of urine are evaporated to a syrupy consistency (to about $\frac{1}{6}$) and thoroughly cooled by immersion in cold water. Excess of concentrated nitric acid is added to the cold solution, the solution being kept cold during the addition and stirred vigorously. Urea nitrate is precipitated in crystalline form. The crystals are filtered off through glass wool, or asbestos, and freed as much as possible from mother liquor by pressing between sheets of paper, or the crystals are

placed on a porous plate and drained from mother liquor. About 4 gm. should be obtained corresponding to about 2 gm. of urea (163 gm. nitrate contain 60 gm. urea). Urea is prepared from the nitrate by mixing it with excess of barium carbonate and adding a little alcohol to form a paste; carbon dioxide is evolved, barium nitrate and urea are formed. The paste is extracted with hot alcohol, the alcohol filtered from barium carbonate and evaporated. Urea separates in needles and is recrystallised from alcohol.

(iii) Urea may also be prepared from urine by precipitating it as urea oxalate by adding 1 gm. of oxalic acid to every 10 c.c. urine; the yield is about 1 per cent. (Roaf).

(II) By synthesis from ammonium cyanate.

A solution of approximately equal parts of ammonium sulphate and potassium cyanate are boiled together and evaporated to dryness on the water-bath. The dry residue is extracted with alcohol, which dissolves the urea leaving potassium sulphate. Urea is left as a residue on evaporating the alcoholic solution on a water-bath. It is crystallised from alcohol :—

$$(NH_4)_2SO_4 + 2KOCN = 2NH_4OCN + K_2SO_4$$
$$NH_4OCN = CH_4ON_2.$$
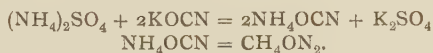
Urea is also formed by heating metallic cyanates, e.g. lead cyanate with water :—

$$Pb(OCN)_2 + 2H_2O = PbCO_3 + CH_4ON_2.$$

(III) By other synthetical methods.

(1) Urea is formed in small quantities by the action of ammonia upon phosgene :—

$$COCl_2 + 2NH_3 = 2HCl + CH_4ON_2.$$

(2) It is formed by the action of ammonia upon ethyl carbonate :—

$$CO(OC_2H_5)_2 + 2NH_3 = 2HOC_2H_5 + CH_4ON_2.$$
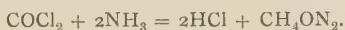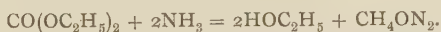
(3) It is formed in small quantities by heating ammonium carbonate :—

$$CO(ONH_4)_2 = H_2O + CO\begin{cases} NH_2 \\ ONH_4 \end{cases} = H_2O + CH_4ON_2.$$

Ammonium Carbamate.
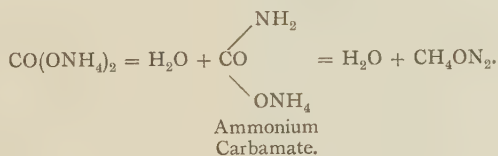
Constitution.

The last three reactions, similar to the methods for preparing amides, indicate that urea should have the constitution of $CO(NH_2)_2$, the amide of carbonic acid. In all these reactions the yield of urea is very small; cyanuric acid, cyamelide, biuret, and ammelide are formed at the same time. On this constitutional basis there is no explanation

for the synthesis of urea from ammonium cyanate except one of molecular rearrangement. Certain other reactions of urea also do not support this constitution, and alternative formulæ for urea have been suggested. The constitution of urea has been specially studied by Werner, who regards urea as having a cyclic formula in neutral solution, but in an acid, or alkaline, solution a formula containing an OH and an NH_2 group, thus

$$HN{=}C\!\!\begin{array}{c} \diagup O \\ \big| \\ \diagdown NH_3 \end{array} \qquad\qquad HN{=}C\!\!\begin{array}{c} \diagup OH \\ \diagdown NH_2. \end{array}$$

In neutral solution.   In acid or alkaline solution.
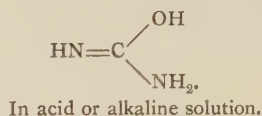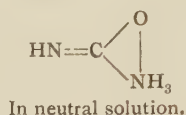
The last formula explains the formation of urea from ammonium cyanate and the composition of salts of urea with one molecule of acid, the decomposition of urea by heat and other reactions. The synthesis of urea from ammonium cyanate, according to Werner, depends upon its dissociation into ammonia and cyanic acid. Cyanic acid changes to isocyanic acid which combines with ammonia to give urea, thus

$$NH_4OCN \rightleftharpoons NH_3 + HOCN$$
$$HOCN \rightleftharpoons HNCO$$

$$HNCO + NH_3 \rightleftharpoons HNC\!\!\begin{array}{c} \diagup OH \\ \diagdown NH_2 \end{array} \quad \text{or } HNC\!\!\begin{array}{c} \diagup O \\ \big| \\ \diagdown NH_3. \end{array}$$
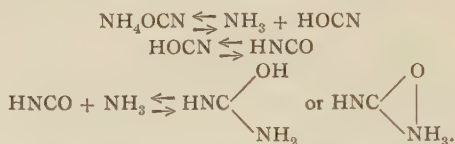
These reactions, most especially that of cyanic acid to isocyanic acid, are reversible. Cyanic acid is more stable at low temperatures, and a rise in temperature favours the formation of isocyanic acid, and hence of urea. These reactions also explain the hydrolysis of urea. Cyanic acid, the nitrile of carbonic acid, is produced and rapidly decomposes as follows :—

$$HOCN \rightarrow HOC\!\!\begin{array}{c} \diagup O \\ \diagdown NH_2 \end{array} \rightarrow HOC\!\!\begin{array}{c} \diagup O \\ \diagdown ONH_4 \end{array} \rightarrow H_2O + CO_2 + NH_3$$

Carbamic acid.   Am. bicarbonate.
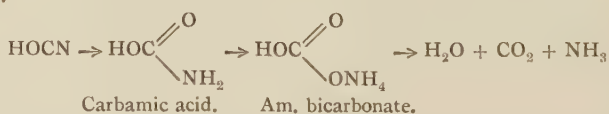
The formation of cyamelide, cyanuric acid, biuret, ammelide, and urethane in the other three reactions for synthesising urea shows that isocyanic and cyanic acids are intermediate products. Urea is always formed by the union of isocyanic acid and ammonia, thus

$$CO\!\!\begin{array}{c} \diagup Cl \\ \diagdown Cl \end{array} + 2NH_3 = NH_4Cl + CO\!\!\begin{array}{c} \diagup NH_2 \\ \diagdown Cl \end{array}$$

Chloroformamide.

$$CO\!\!\begin{array}{c} \diagup NH_2 \\ \diagdown Cl \end{array} = HNCO + HCl \qquad HNCO \rightleftharpoons HOCN$$

$$HNCO + NH_3 = HNC\begin{matrix} \diagup OH \\ \diagdown NH_2 \end{matrix}$$

$$CO\begin{matrix} \diagup OC_2H_5 \\ \diagdown OC_2H_5 \end{matrix} + NH_3 = CO\begin{matrix} \diagup NH_2 \\ \diagdown OC_2H_5 \end{matrix} + HOC_2H_5$$

Urethane.

$$CO\begin{matrix} \diagup NH_2 \\ \diagdown OC_2H_5 \end{matrix} = HNCO + HOC_2H_5 \qquad HNCO \rightleftharpoons HOCN$$

$$HNCO + NH_3 = HNC\begin{matrix} \diagup OH \\ \diagdown NH_2 \end{matrix}$$

$$CO\begin{matrix} \diagup ONH_4 \\ \diagdown ONH_4 \end{matrix} \rightarrow CO\begin{matrix} \diagup NH_2 \\ \diagdown ONH_4 \end{matrix} \rightarrow CO\begin{matrix} \diagup NH_2 \\ \diagdown OH \end{matrix} + NH_3$$

Am. carbonate. Am. carbamate. Carbamic acid.

$$CO\begin{matrix} \diagup NH_2 \\ \diagdown OH \end{matrix} \rightarrow HNCO + H_2O \qquad HNCO \rightleftharpoons HOCN$$

$$HNCO + NH_3 = HNC\begin{matrix} \diagup OH \\ \diagdown NH_2 \end{matrix}$$

The formation of the other products in these reactions, which are known to arise from cyanic, or isocyanic acid, is also explained :—

3(HOCN) → cyamelide at low temperatures,
3(HNCO) → cyanuric acid at high temperatures,
urea + HNCO → biuret,
biuret + HNCO → ammelide.
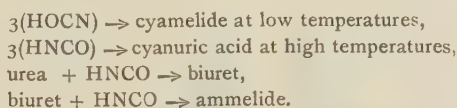
Synthesis of Urea in the Animal Organism.

Ammonia is produced in the body by decomposition of the nitrogenous foodstuffs, proteins. It is converted by the liver, and to a lesser extent, by other tissues, into urea. Ammonium carbonate will be first produced; by loss of water, it passes into urea, by the same series of reactions as given above. The presence of cyanic, or isocyanic, acid in blood and tissues has not yet been definitely proved; the last stage in the reaction is probably so rapid that its presence is difficult to show.
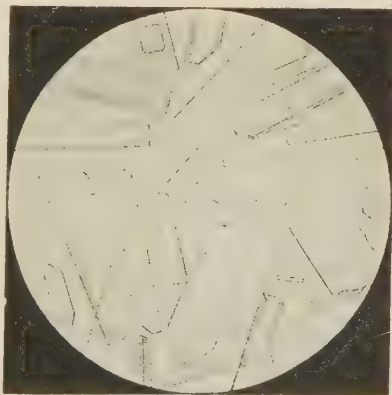
FIG. 19.—Urea. (After Funke.)

Properties and Reactions.

Urea is a white solid which crystallises from water in long prisms (Fig. 19). It melts at 132° and is easily soluble in water, alcohol, acetone, but not in ether or chloroform.

(1) Urea is a weak base and forms salts with strong acids.

(*a*) *Urea nitrate.*—If a few crystals of urea be dissolved in water on a watch glass and one or two drops of concentrated nitric acid be added, crystals of urea nitrate are formed. These are seen to consist of rhombic six-sided platelets often imbricated (like tiles) when examined under the microscope (Fig. 20).

$$HNC{\Large<}^{OH}_{NH_2} \ + HNO_3 = HNC{\Large<}^{OH}_{NH_2 . HNO_3}$$
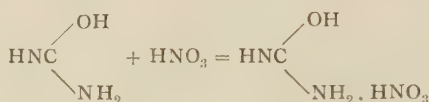
(*b*) *Urea oxalate.*—If a saturated solution of oxalic acid be used
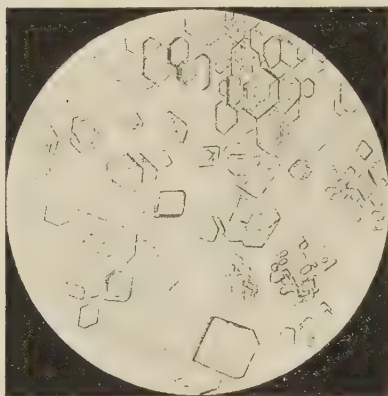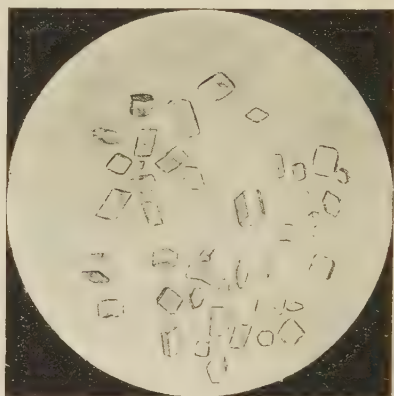
FIG. 20.—Urea nitrate.   FIG. 21.—Urea oxalate.

(After Funke.)

instead of nitric acid, crystals of urea oxalate are formed. Under the microscope they are seen to consist of short rhombic prisms (Fig. 21).

$$2HNC{\Large<}^{OH}_{NH_2} \ + {\ }^{COOH}_{COOH} \ = \ \begin{matrix} COOH . H_2N{\Large\diagdown} \\ HO{\Large\diagup}{\normalsize CNH} \\ \\ COOH . H_2N{\Large\diagdown} \\ HO{\Large\diagup}{\normalsize CNH.} \end{matrix}$$
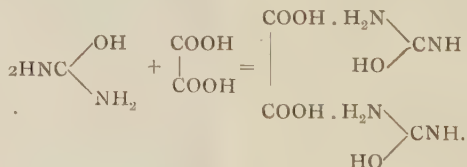
(2) *Action of Sodium Hydroxide.*—Urea is decomposed with evolution of ammonia by boiling its solution with excess of caustic soda :—

$$HNC(OH)NH_2 + 2H_2O = CO_2 + H_2O + 2NH_3.$$
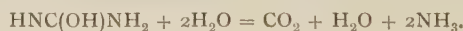
(3) *Action of Nitrous Acid.*—On adding dilute hydrochloric acid and a few drops of sodium nitrite solution to some urea solution, an effervescence of nitrogen and carbon dioxide takes place :—

$$HNC(OH)NH_2 + 2HNO_2 = CO_2 + 3H_2O + 2N_2.$$

(4) *Action of Hypobromite.*—Urea is decomposed into carbon dioxide and nitrogen on adding sodium hypobromite to its solution :—

$$HNC(OH)NH_2 + 3NaOBr = CO_2 + N_2 + 3NaBr + H_2O$$
$$CO_2 + 2NaOH = Na_2CO_3 + H_2O.$$
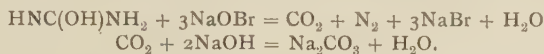
Note.—An effervescence also occurs when sodium hypobromite is added to ammonium chloride.

(5) By adding dilute mercuric nitrate solution (1 per cent.), urea is precipitated from the solution as a white compound having the composition $HNC(OH)NH_2 . Hg(NO_3)_2 . HgO$.

Biuret.

Urea is decomposed on heating with evolution of ammonia and production of biuret, cyanuric acid, and other products. The first stage in the reaction consists in the formation of isocyanic acid and ammonia :—

$$HNC(OH)NH_2 = HNCO + NH_3.$$

Biuret then arises by combination of urea and isocyanic acid :—
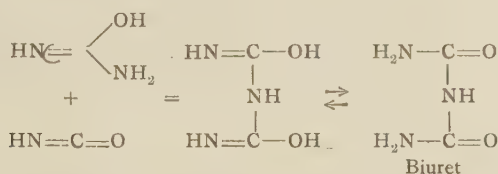
The product of combination appears to change into a tautomeric form.

Biuret crystallises from water in long needles which melt at 193° with decomposition. Its chief characteristic is the biuret reaction, a pink, or violet pink, colour with copper sulphate in alkaline solution.

On heating some urea in a test tube it melts; on continuation of the heating the mass becomes solid, white, and opaque : ammonia is evolved and a ring of sublimed cyanuric acid may be formed on the cooler parts of the test tube. The white residue consists mainly of biuret, but contains also cyanuric acid; on treating the mass with water the biuret dissolves. On pouring off the water and testing it with caustic soda and 1 or 2 drops of dilute copper sulphate (1 per cent.) a pink colour is formed (biuret reaction). The residue is tested for cyanuric acid (see below).

$$\text{Urethane, } HN = C \Big\langle {OH \atop OC_2H_5} \quad \text{or} \quad O = C \Big\langle {NH_2 \atop OC_2H_5}.$$
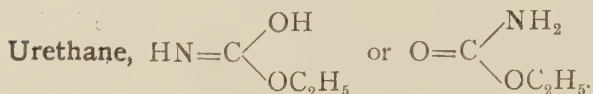
Urethane, the ethyl ester of carbamic acid, is produced by the action of ammonia upon ethyl carbonate, or upon chloroformic ester :—

$$CO \Big\langle {OC_2H_5 \atop OC_2H_5} + NH_3 = CO \Big\langle {NH_2 \atop OC_2H_5}$$

$$CO \Big\langle {Cl \atop OC_2H_5} + NH_3 = CO \Big\langle {NH_2 \atop OC_2H_5}$$
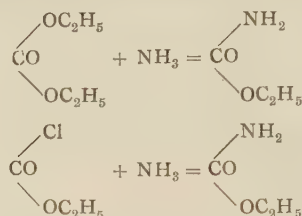
It is formed by the combination of cyanic acid with alcohol :—

$$HOCN \rightarrow HNCO + C_2H_5OH = HN = C \Big\langle {OH \atop OC_2H_5}$$
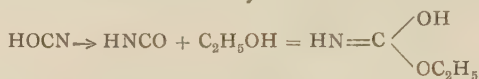
This reaction explains its usual preparation by heating urea with alcohol.

Urethane is a colourless crystalline compound melting at 49° very easily soluble in water.   It is used as a hypnotic.

The Estimation of Urea.

Urea is hydrolysed by water, acids, or alkalies into carbonic acid and ammonia ; it is decomposed by alkaline hypobromite into carbon dioxide and nitrogen.   These reactions are used to determine the amount of urea in a given solution.

*   (1) **Hypobromite Method.**

The most simple and rapid method of estimating urea is the decomposition by alkaline hypobromite ; the carbon dioxide is absorbed by the alkali and the evolved nitrogen is collected and measured.   This method is the one suggested by Hüfner and most frequently employed in medical practice.   The estimation is generally carried out in the apparatus designed by Dupré and known as Dupré's ureometer (Fig. 22).

The estimation is carried out as follows :—

25 c.c. of freshly-prepared hypobromite solution[1] are placed in the bottle of about 120 c.c. capacity.   5 c.c. of urea solution are measured out with a pipette into a small tube which is placed in the bottle, taking great care not to upset the solution into the hypobromite.   The bottle is closed with an india-rubber stopper and placed in cold water to cool. Through the india-rubber stopper a glass T-piece passes.   One end

---

[1] 100 gm. caustic soda are dissolved in 250 c.c. water and to the cold liquid 25 c.c. bromine are added.

of this is connected by rubber tubing to a graduated burette which is placed in a jar of water. The rubber tubing is of such a length that the burette can be lifted out of the water without stretching. The other end of the T-piece is closed by a piece of rubber tubing and a small clip. The burette is filled with water by opening the screw clip; and it is raised, or lowered, until the water stands at the uppermost graduation and at the same level outside and inside. The clip is closed and leakage in the system is tested for by raising and lowering the burette in the water for at least a minute and then seeing whether the level of the water returns to the top graduation when the water inside and outside the tube are again made to stand at the same level. When the system has been tested to see that it is air-tight, the analysis can be commenced. The reading of the top graduation is noted. The bottle is tilted so as to upset the urea solution in the little tube into the hypobromite solution and it is thoroughly washed out with the latter. Nitrogen is rapidly evolved and displaces the water in the burette. The bottle must now be brought to its original temperature by placing it for a few minutes in a fresh supply of cold water. As soon as it is cool, the burette is raised till the level of the water is the same inside and outside and the level is read. The difference in the readings gives the volume of nitrogen evolved.
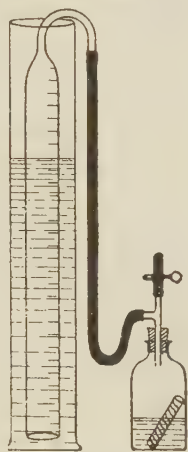
FIG. 22.

By making the levels inside and outside the burette the same, this volume is measured at the atmospheric pressure. The temperature of the water and the barometric pressure and the tension of aqueous vapour at that temperature are ascertained and the volume of nitrogen is corrected to the volume at 0° and 760 mm. by the formula :—

$$\frac{V \times 273 \times (B - T)}{(273 + t) \times 760}$$

where V = volume of gas evolved, B = barometric pressure, T = tension of aqueous vapour.

The amount of urea corresponding to this volume is given by the equation :—

$$HNC(OH)NH_2 + 3NaBrO + 2NaOH = 3NaBr + N_2 + Na_2CO_3 + 3H_2O.$$

60 gm.   22·4 litres (= 28 gm.)
1 gm.   373 c.c.
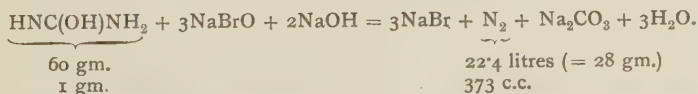
from which the amount of urea in 5 c.c. of the solution is calculated; hence the amount in 100 or 1000 c.c.

Actually, however, only 354 c.c. nitrogen are evolved by 1 gm. of urea so that the method is not quite accurate ; this is not usually allowed for.   Rapidity is the chief advantage of this method.   The results are sufficiently accurate for ordinary medical requirements.

(2) By Hydrolytic Methods.

The most accurate methods of estimating urea are by hydrolysis. Urea is rapidly hydrolysed by alkali, but more slowly by hydrochloric acid.

(i) *By Acid.*

The hydrolysis by acid proceeds rapidly and is complete in about 1 hour if the hydrolysis be effected at a temperature of about 150-160° as was shown by Folin.   This method has been particularly useful in the analysis of urine.

(ii) *By Urease.*

Since the discovery of the enzyme, urease, in the soy bean which converts urea into ammonia and carbon dioxide, this hydrolytic agent has displaced hydrochloric acid on account of simplicity.   The action of urease upon urea is to catalyse the change into isocyanic acid and ammonia :—

$$HNC{\Large<}^{OH}_{NH_2} = HNCO + NH_3.$$
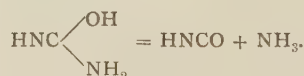
Isocyanic acid changes to cyanic acid which decomposes into carbon dioxide and ammonia.

$$HNCO \rightarrow HOCN + H_2O = CO_2 + NH_3.$$

An apparatus consisting of a wash bottle, a gas cylinder or other tall vessel, and a receiving bottle is required (Fig. 23).   The gas cylinder is fitted with a cork carrying a long tube reaching to the bottom and a short tube ; the receiving bottle is fitted with a cork carrying a tube with a bulb blown at its end and pierced with several small holes.   The long tube in the gas cylinder is connected by rubber tubing to the wash bottle and the short tube to the bulb tube of the receiving bottle.   The gas cylinder is placed in warm water at 40°, or in a bath kept at 40°, to hasten the decomposition of the urea.

The wash bottle contains sulphuric acid to remove ammonia from the air drawn through the vessels.   The receiving bottle contains a known volume (25 or 50 c.c.) of ·1 N sulphuric acid coloured with a few drops of alizarin red, or methyl orange, solution.   The solution in the receiving bottle must amply cover the bulb.

Air is drawn as rapidly as possible through the apparatus by suction with a pump attached to the short tube of the receiving bottle. 5 c.c. of the solution of urea (1-2 per cent.), or urine, 25 c.c. of water, and about 2 c.c. of kerosene, or liquid paraffin, and 0·5-1 gm. of powdered soy bean are placed in the gas cylinder, and air is drawn through the apparatus for $\frac{1}{2}$-1 hour depending on the amount of urea present. At the end of this time the parts of the apparatus are disjointed, 1 gm. of anhydrous sodium carbonate put into the gas cylinder so as to liberate ammonia which may be retained as ammonium salt, the connections are again made and the ammonia drawn into the receiv-

FIG. 23.

ing bottle by air suction for another half-hour. The excess of ·1 N acid in the bottle is titrated with ·1 N alkali. The amount of urea is calculated from the equation :—

$$HNC(OH)NH_2 + H_2O = CO_2 + 2NH_3$$

$$\underbrace{}\qquad\qquad\underbrace{}$$

60   34
30   17
1 c.c. of ·1 N alkali = 0·003 gm. urea.
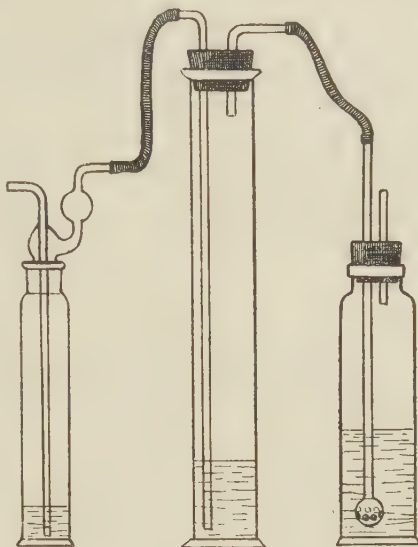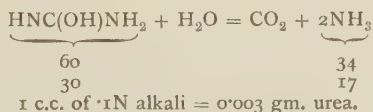
Though this method takes longer and requires more apparatus and a good suction pump, the results are accurate. It is used in all scientific investigations.

Cyanuric Acid and Cyamelide, $C_3H_3O_3N_3$.

Cyanuric acid is formed on heating urea, most conveniently by heating 1 part of urea with 2 parts of zinc chloride. Its formation depends upon the reversion of urea into isocyanic acid and ammonia. Isocyanic acid then polymerises to cyanuric acid :—

$$3HNCO = HN{=}C\begin{matrix} O{-}C{=}NH \\ \diagup \qquad\qquad \diagdown \\ \qquad\qquad O \\ \diagdown \qquad\qquad \diagup \\ O{-}C{=}NH \end{matrix}$$
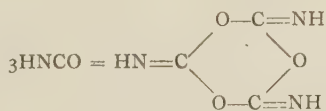
Cyanuric acid crystallises from water with 2 molecules of water of crystallisation in large rhombic prisms. It is soluble in 40 parts of cold

water, more easily in hot water and alcohol. It is decomposed by boiling with acids into carbon dioxide and ammonia.

It is a tribasic acid forming soluble salts with the alkali metals and insoluble salts with the heavy metals ; the copper salt is violet in colour.

The residue (p. 127) dissolves in dilute ammonia ; if barium chloride be added to a portion of this solution a white precipitate of barium cyanurate is formed ; on adding copper sulphate to another portion, an amethyst-coloured precipitate of copper cyanurate is formed.

Cyamelide is formed by the polymerisation of cyanic acid :—

$$3HOCN = HO—C \begin{array}{c} N—C—OH \\ \diagup \diagdown \\ \diagdown \qquad N \\ \diagup \\ N = C—OH \end{array}$$
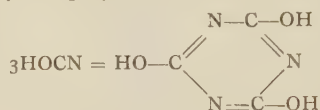
Ammelide is formed in the various reactions connected with urea by combination of biuret with isocyanic acid :—

$$HN \begin{array}{c} CO—NH_2 \\ \diagup \\ \diagdown \\ CO—NH_2 \end{array} + OCNH = HN \begin{array}{c} CO—NH \\ \diagup \qquad \diagdown \\ \diagdown \qquad \diagup \\ CO—NH \end{array} C = NH + H_2O$$
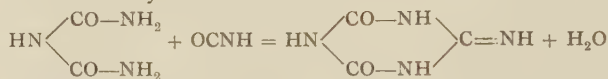
Alkyl Isocyanates.

The existence of alkyl cyanates has not been established.

Alkyl isocyanates are formed on heating silver cyanate with an alkyl iodide :—

$$AgNCO + CH_3I = CH_3NCO + AgI.$$
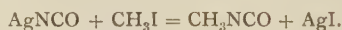
These compounds are also obtained when potassium cyanate is distilled with alkyl potassium sulphate :—

$$KOCN + CH_3O . SO_2 . OK = CH_3 . NCO + KO . SO_2 . OK.$$
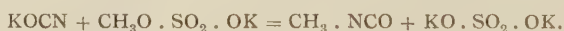
A rearrangement of the atoms of the molecule has occurred on heating. They are also formed from amides (p. 109).

The alkyl isocyanates are volatile liquids with a strong, disagreeable, suffocating odour. They boil without decomposition and are soluble in ether. They polymerise on standing forming isocyanuric esters.

Their structure is shown by their conversion into amines by the action of potash :—

$$CH_3 . NCO + H_2O = CH_3 . NH_2 + CO_2.$$
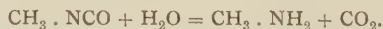
Cyanogen Chloride and Cyanogen Bromide, ClCN.

If mercuric cyanide, potassium cyanide, or hydrogen cyanide be treated with chlorine or bromine, cyanogen chloride or bromide is obtained :—

$$KCN + Cl_2 = ClCN + KCl.$$
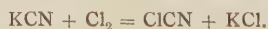
These compounds are liquids with pungent smells. They polymerise on standing into solid cyanuric chloride $Cl_3C_3N_3$ or bromide $Br_3C_3N_3$.

Potash converts cyanogen chloride into potassium cyanate, and solid cyanuric chloride into potassium cyanurate :—

$$ClCN + 2KOH = KOCN + KCl + H_2O.$$
$$Cl_3C_3N_3 + 6KOH = K_3O_3C_3N_3 + 3KCl + 3H_2O.$$

Cyanamide, $NH_2 . CN$.

Cyanamide is formed on passing cyanogen chloride into an ethereal or aqueous solution of ammonia :—

$$ClCN + NH_3 = NH_2 . CN + HCl.$$

It is more readily obtained by the action of mercuric oxide on thiourea :—

$$CS{<}{\begin{array}{c}NH_2\\NH_2\end{array}} + HgO = HgS + H_2O + NH_2 . CN.$$
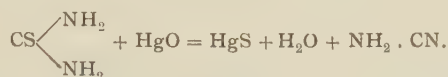
Calcium cyanamide is manufactured by passing nitrogen over calcium carbide at 1000° :—

$$CaC_2 + N_2 = CaCN_2 + C.$$
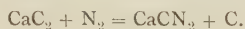
Cyanamide is prepared from the salt by decomposition with aluminium sulphate, filtration, evaporation *in vacuo* and crystallisation from ether.

Sodium cyanamide is prepared as described on p. 116.

Cyanamide forms colourless hygroscopic crystals, easily soluble in water, alcohol, and ether, and melting at 40°. It forms salts with strong acids and also with bases. The salts with acids are decomposed by water. The calcium salt is frequently employed as an artificial manure.

Cyanamide is readily hydrolysed by the action of acids forming urea :

$$H_2N . CN + H_2O = H_2N{-}C{<}{\begin{array}{c}NH\\OH\end{array}}$$
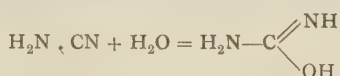
By the action of hydrogen sulphide it is converted into thiourea, and by ammonia it is converted into guanidine.

Cyanamide is used in the synthesis of creatine (p. 302), and arginine (p. 301), compounds containing a guanidine nucleus. These compounds behave like cyanamide in giving urea on hydrolysis.

Thiocyanic Acid, $HSCN$.

Thiocyanic acid, or sulphocyanic acid, has long been known to be present in the form of its salts in saliva and it has also been found in other secretions of the animal body. The amount is always very small.

Thiocyanic acid is obtained by distilling its potassium salt with dilute sulphuric acid, or by the action of dry hydrogen sulphide upon mercuric thiocyanate.

Thiocyanic acid is a gas and, like cyanic acid, is easily condensed in a freezing mixture to a liquid which has a penetrating and acrid odour and is soluble in water and alcohol. It is an unstable substance ;

on removal from the freezing mixture it polymerises to a yellow amorphous body.   It forms soluble salts with the alkali metals and insoluble salts with the heavy metals.

Potassium Thiocyanate.

Potassium thiocyanate is readily prepared from potassium cyanide by evaporating its solution with flowers of sulphur or ammonium sulphide : thus :—

10 c.c. of a 1 per cent. solution of potassium cyanide are boiled for some minutes with flowers of sulphur and filtered.   The presence of potassium thiocyanate is shown by the red colour which is formed on the addition of a drop of ferric chloride solution.

Potassium thiocyanate crystallises from alcohol in long colourless prisms, which deliquesce in the air.

Sodium thiocyanate is also deliquescent.

Ammonium thiocyanate is prepared in a similar manner to the potassium salt.   It is a product obtained in the manufacture of coal gas from ammonium salts, hydrogen cyanide, and sulphur.

It is usually prepared by the action of carbon bisulphide upon alcoholic ammonia, or ammonia under pressure.   Ammonium thiocarbamate is formed, and this is decomposed by steam into ammonium thiocyanate and hydrogen sulphide :—

$$2NH_3 + CS_2 = CS \begin{cases} NH_2 \\ S \cdot NH_4. \end{cases}$$

$$CS \begin{cases} NH_2 \\ S \cdot NH_4 \end{cases} = H_2S + NH_4SCN.$$
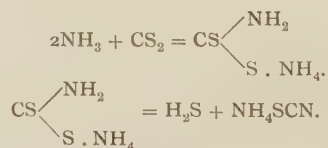
Ammonium thiocyanate crystallises in prisms which are easily soluble in water and alcohol.

Metallic Thiocyanates.

(1) Ferric thiocyanate is formed on adding ferric chloride solution to a soluble thiocyanate ; ferric thiocyanate is soluble and has an intense red colour and is used in detecting thiocyanates.

(2) Silver thiocyanate is thrown down as a white curdy precipitate on adding silver nitrate to a solution of a thiocyanate.

Alkyl Thiocyanates and Alkyl Isothiocyanates.

Alkyl thiocyanates are prepared by the action of alkyl halides upon potassium thiocyanate :—

$$KSCN + C_2H_5I = C_2H_5SCN + KI.$$
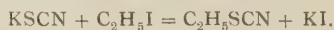
Alkyl isothiocyanates, or mustard oils, are obtained by heating alkyl thiocyanates, isomeric change occurring :—

$$C_2H_5SCN \rightarrow C_2H_5NCS.$$

They are also prepared by the action of primary amines on carbon bisulphide in alcoholic, or ethereal, solution, and then heating the aqueous solution of the thiocarbamate so formed with mercury chloride, or ferric chloride :—

$$CS_2 + 2C_2H_5 . NH_2 = CS \begin{cases} NH . C_2H_5 \\ SH . NH_2 . C_2H_5 \end{cases}$$

$$CS \begin{cases} NH . C_2H_5 \\ SH . NH_2 . C_2H_5 \end{cases} = H_2S + NH_2 . C_2H_5 + C_2H_5NCS.$$
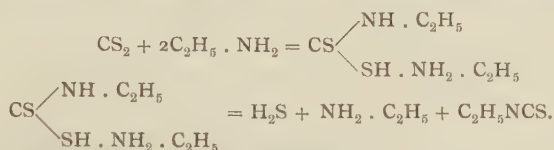
The alkyl isothiocyanate distils over with steam.

The alkyl thiocyanates are oily liquids, insoluble in water, possessing a garlic-like smell.

The alkyl isothiocyanates are pungent smelling liquids, the odour of which provokes tears. They are generally called mustard oils on account of the occurrence of allyl isothiocyanate in mustard. They boil at a lower temperature than the isomeric alkyl thiocyanates and are almost insoluble in water.

Allyl isothiocyanate occurs in combination in the glucoside, sinigrin, of mustard seed. The allyl thiocyanate is formed on contact with water, the glucoside being decomposed by the enzyme, myrosin. To the allyl thiocyanate is due the pungent smell and taste of mustard.

Constitution of Alkyl Thiocyanates and Isothiocyanates.

In the thiocyanates the alkyl group is joined to the sulphur atom; in the isothiocyanates it is attached to the nitrogen atom as is shown by the following reactions :—

(1) Thiocyanates on reduction give the primary amine and a mercaptan :—

$$C_2H_5SCN + 3H_2 = C_2H_5SH + CH_3NH_2.$$
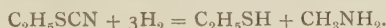
(2) Thiocyanates on oxidation give a sulphonic acid :—

$$C_2H_5SCN + O \rightarrow C_2H_5SO_3H.$$
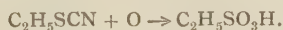
(3) Thiocyanates on treatment with alcoholic potash give alcohol and potassium thiocyanate :—

$$C_2H_5SCN + KOH = KSCN + C_2H_5OH.$$

These reactions point to the attachment of the alkyl group to the sulphur atom.

(1) Isothiocyanates on heating with hydrochloric acid yield amines :—

$$C_2H_5NCS + 2H_2O = C_2H_5NH_2 + CO_2 + H_2S.$$

(2) Isothiocyanates on reduction yield a primary amine and thioformaldehyde :—

$$C_2H_5NCS + 2H_2 = C_2H_5NH_2 + HCSH.$$
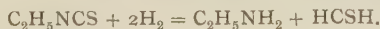
(3) Isothiocyanates are converted into isocyanates by boiling their solution in alcohol with mercuric oxide, or chloride :—

$$C_2H_5NCS + HgO = C_2H_5NCO + HgS.$$
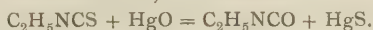
The alkyl group is thus attached to the nitrogen atom.

HYDROXY-, KETO-, AND DIBASIC ACIDS.

In the previous chapters compounds having only a single function of either alcohol, aldehyde, or acid have been considered. In compounds of carbon containing two or more atoms of carbon in their molecule the replacement of hydrogen atoms by other atoms or groups can occur in several of the atoms, and compounds will result which have multiple functions. Two or more alcohol groups, attached to different carbon atoms, may be present in a molecule. Such compounds are the poly-hydric alcohols (see p. 231). Hydroxy acids have an alcohol group and a carboxyl group, ketonic or keto-acids a ketone group and an acid group. Dibasic acids contain two carboxyl groups and so on. Numerous natural compounds are included amongst the large number of compounds which are theoretically possible : most, if not all, of these have been prepared in the laboratory by the same methods which are used in making the simple compound. The properties of such compounds are the sum of the properties possessed by the particular groups contained in the molecule.

COMPOUNDS CONTAINING TWO CARBON ATOMS.

The variety of the compounds is most easily seen in the series of compounds which are derived from ethane :—

CH_3	CH_2OH	CHO	$COOH$	CHO	$COOH$	$COOH$
CH_3	CH_2OH	CH_2OH	CH_2OH	CHO	CHO	$COOH$
Ethane.	Glycol.	Glycollic aldehyde.	Glycollic acid.	Glyoxal.	Glyoxylic acid.	Oxalic acid.

Glycol.

Glycol is the first member of the series of polyhydric alcohols, and is prepared from ethylene dibromide :—

$$\begin{array}{l} CH_2Br \\ | \\ CH_2Br \end{array} + 2KOH = 2KBr + \begin{array}{l} CH_2OH \\ | \\ CH_2OH. \end{array}$$
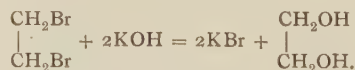
Glycol may also be obtained directly from ethylene by oxidation with permanganate (p. 47).

Glycol is a colourless syrupy liquid with a sweet taste; it boils at 198° and has a sp. gr. of 1·1297 at 0°. It is miscible with water and alcohol in all proportions, but is very slightly soluble in ether. It is very hygroscopic and takes up water from the atmosphere forming a hydrate, $C_2H_6O_2 . 2H_2O$.

Glycol forms a mono-sodium, or di-sodium derivative, with metallic sodium, from which the corresponding ethers may be prepared. Esters are produced according to the proportion of acid which is used in the reaction. Both hydroxyl groups are replaced by Cl on treatment with phosphorus pentachloride. Hydrochloric acid usually replaces only one OH group, yielding the chlorhydrin :—

$$\begin{matrix} CH_2OH \\ | \\ CH_2OH \end{matrix} + HCl = \begin{matrix} CH_2OH \\ | \\ CH_2Cl \end{matrix} + H_2O.$$
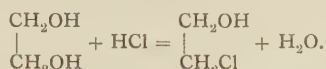
This compound is also obtained by the addition of hypochlorous acid to ethylene.

Ethylene Oxide.

Glycol chlorhydrin on treatment with alkali, yields ethylene oxide :—

$$\begin{matrix} CH_2OH \\ | \\ CH_2Cl \end{matrix} + KOH = \begin{matrix} CH_2 \\ | \\ CH_2 \end{matrix}\Big\rangle O + KCl + H_2O.$$
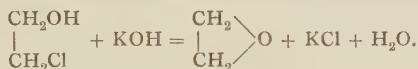
Ethylene oxide is a liquid boiling at 12°. It is a very reactive compound forming derivatives by addition, e.g.—

$$\begin{matrix} CH_2 \\ | \\ CH_2 \end{matrix}\Big\rangle O + H_2 = \begin{matrix} CH_3 \\ | \\ CH_2OH \end{matrix} \qquad \begin{matrix} CH_2 \\ | \\ CH_2 \end{matrix}\Big\rangle O + H_2O = \begin{matrix} CH_2OH \\ | \\ CH_2OH \end{matrix}$$

$$\begin{matrix} CH_2 \\ | \\ CH_2 \end{matrix}\Big\rangle O + HCl = \begin{matrix} CH_2Cl \\ | \\ CH_2OH \end{matrix} \qquad \begin{matrix} CH_2 \\ | \\ CH_2 \end{matrix}\Big\rangle O + NH_3 = \begin{matrix} CH_2 . NH_2 \\ | \\ CH_2OH \end{matrix}$$
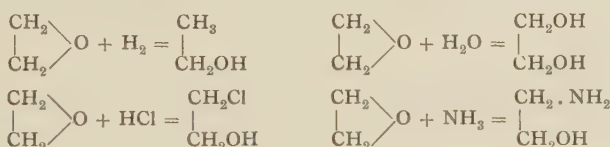
Glycollic Aldehyde.

Glycollic aldehyde is prepared by oxidising glycol with hydrogen peroxide in the presence of ferrous sulphate, or by hydrolysing bromacetaldehyde with baryta.

It is a sweet crystalline substance having the properties of an aldehyde. It is the first representative of the group of carbohydrates (p. 249).

Glycollic Acid.

Glycollic acid is prepared by boiling potassium chloracetate under a reflux condenser with water :—

$$CH_2Cl . COOK + H_2O = CH_2OH . COOH + KCl.$$
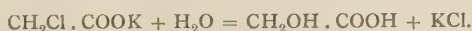
The solution is evaporated *in vacuo* to dryness and the glycollic acid extracted from the residue with acetone.

10

It is present in unripe fruit and was first obtained from glycine (p. 171):—

$$CH_2.NH_2 \atop COOH + HNO_2 = {CH_2OH \atop COOH} + N_2 + H_2O.$$
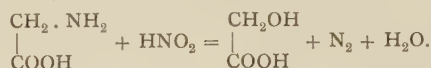
It can also be obtained from formaldehyde cyanhydrin :—

$$HC{\overset{H}{\underset{CN}{<}}}{\overset{OH}{}} + 2H_2O = {CH_2OH \atop COOH} + NH_3.$$
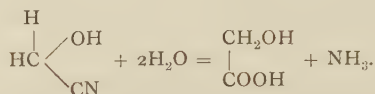
Glycollic acid is a deliquescent crystalline solid which melts about 80°. It is a monobasic acid and at the same time a primary alcohol, and consequently has the properties of both of these types of compounds.

Glyoxal.

Glyoxal can be prepared by oxidising acetaldehyde with nitric acid at the ordinary temperature and is isolated as its bisulphite compound.

It is an amorphous solid, or when not quite free from water, a syrup. It has all the properties of an aldehyde.

Glyoxylic Acid.

Preparation.

Glyoxylic acid is prepared most conveniently by the reduction of oxalic acid; sodium amalgam was most frequently employed until Benedict suggested the use of magnesium.

* About 1 gm. of powdered magnesium is placed in a small flask and just covered with distilled water; 25 c.c. of saturated oxalic acid solution are slowly added. The reaction proceeds rapidly with liberation of heat and the flask should be cooled with water. The insoluble magnesium oxalate, which is formed, is filtered off and the glyoxylic acid is obtained by evaporation *in vacuo.*

Properties.

Glyoxylic acid is a syrup, very soluble in water. It gives the reactions of an acid and of an aldehyde.

* The solution, prepared above, may be tested for aldehyde by Schiff's reaction, ammoniacal silver nitrate and other reactions. It is used in testing for proteins, the above solution being acidified with acetic acid and made up to 100 c.c. with distilled water.

Oxalic Acid.

Oxalic acid occurs naturally in the form of its acid potassium salt in many plants—e.g. sorrel, rhubarb. It is also deposited in the cells

of plants as calcium oxalate.   It is produced from sugar by many bacteria and moulds.

Small quantities of oxalic acid are present in normal urine, from ·02-·12 gm. in 24 hours.   It arises most probably from the carbohydrate of the diet.   An increased output follows the consumption of rhubarb and other vegetables which contain oxalic acid, and occasionally an increased output occurs in certain diseases, e.g. in diabetes.   Calculi of calcium oxalate are sometimes found in the bladder and kidneys.

Preparation.

* Oxalic acid is formed by the oxidation of numerous organic compounds, especially acetic acid and sugar.   5 gm. of cane sugar are heated carefully in a large flask with 18-20 c.c. strong nitric acid in a fume cupboard.   After the evolution of brown fumes has ceased, the solution is evaporated to about a quarter.   Oxalic acid crystallises out on cooling.

It is made commercially by the oxidation of the cellulose of sawdust with air and caustic alkali.   A mixture of caustic potash and caustic soda is made into a paste with sawdust and heated in open vessels to about 240°.   The mass is extracted with cold water ; the potash dissolves leaving sodium oxalate which is only slightly soluble. By boiling the sodium oxalate with milk of lime, insoluble calcium oxalate is formed.   This is washed and decomposed with sulphuric acid, and the oxalic acid isolated from the solution by crystallisation.

The alkali salts of oxalic acid are made commercially by heating alkali formates.   The reaction proceeds most easily in the presence of small amounts of alkali at 280° under diminished pressure, or at 400° in absence of air :—

$$2HCOONa = H_2 + \begin{matrix} COONa \\ | \\ COONa. \end{matrix}$$
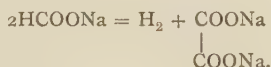
Properties.

* Oxalic acid crystallises from water in colourless prisms containing 2 molecules of water of crystallisation (m.p. 101·5°).   On heating to 100°, it loses the water, becomes opaque, and forms a white powder which melts at 189°.

It is easily soluble in alcohol, but only slightly soluble in ether. It is insoluble in chloroform, benzene, and petroleum ether.

Reactions.

* (1) On heating on platinum, nickel, or a crucible lid, oxalic acid is volatilised without charring.

10 *

* (2) No charring occurs on heating oxalic acid with concentrated sulphuric acid, but it. is decomposed yielding carbon monoxide and carbon dioxide :—

$$COOH . COOH = CO_2 + CO + H_2O.$$
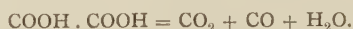
The gases may be passed into lime or baryta water ; barium carbonate is precipitated and the carbon monoxide may be ignited.

* (3) On warming a solution of oxalic acid with dilute sulphuric acid and potassium permanganate, it is oxidised with liberation of carbon dioxide and the permanganate is decolorised :—

$$COOH . COOH + O = 2CO_2 + H_2O.$$
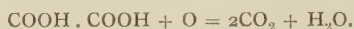
* (4) Calcium oxalate is precipitated when calcium chloride is added to a solution of a *neutral* oxalate. It is insoluble in acetic acid, but soluble in mineral acids.

(5) Mercurous nitrate gives a precipitate of mercurous oxalate even in very dilute solutions of *neutral* oxalates.

Detection.

* Oxalic acid is precipitated from a neutral solution, or a solution acidified with acetic acid as calcium oxalate. The crystalline form of the precipitate (p. 531) is very characteristic. The calcium oxalate is filtered off and can be identified by heating it, or by the action of permanganate in sulphuric acid solution.

Salts of Oxalic Acid.

As a dibasic acid, oxalic acid forms two series of salts :—

$$\begin{array}{ccc} COOK & COOK & COO\diagdown \\ | & | & | \quad\diagup Fe \\ COOK & COOH & COO\diagup \end{array}$$

Acid potassium oxalate combines with a molecule of oxalic acid forming potassium quadroxalate. Under the name of salts of lemon, or salts of sorrel, it is used to remove inkstains and iron moulds. Ferrous oxalate is precipitated as a yellow powder on adding ferrous sulphate to a neutral oxalate. Potassium ferrous oxalate has powerful reducing properties and is used as a developer in photography.

Esters of Oxalic Acid.

Two series of esters can be prepared. Methyl oxalate is solid (p. 59). The acid esters are soluble in water.

Oxamide.

Preparation.

* Oxamide is precipitated on adding excess of ammonia to ethyl oxalate (1 c.c.). The precipitate is filtered off, washed with water, and dried.

Properties and Reactions.

Oxamide is a white solid almost insoluble in water.

* On boiling it for some time with caustic soda, it gradually dissolves forming sodium oxalate and at the same time ammonia is evolved.

COMPOUNDS CONTAINING THREE CARBON ATOMS.

The number of compounds which can be derived from propane are more numerous than those from ethane, since the extra carbon atom introduces further possible combinations and permutations. The principal compounds are :—

CH_2OH	CH_3	CH_3	CH_3	COOH	COOH	COOH
CH_2	CHOH	CO	CO	CH_2	CHOH	$C(OH)_2$
COOH	COOH	COOH	CHO	COOH	COCH	COOH
β-hydroxy-propionic acid.	α-hydroxy-propionic acid.	Pyruvic acid.	Methyl glyoxal.	Malonic acid.	Tartronic acid.	Mesoxalic acid.
Hydracrylic acid.	Lactic acid.					
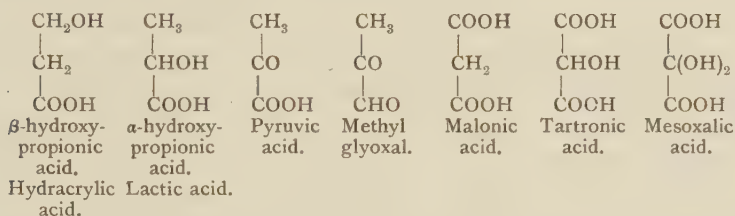
The first two compounds are isomers ; in their nomenclature the position of the hydroxyl group is indicated by the Greek letters a and β, the lettering, or numbering, being commenced at the carbon atom next to the COOH group, which stands at the end of the chain (cf. p. 100).

β-Hydroxypropionic Acid.

The chief interest in this compound is with reference to its isomerism with lactic acid. It contains an ethylene radicle and is sometimes also known as ethylene lactic acid. This is shown by its synthesis :—

CH_2	HOCl	CH_2OH	KCN	CH_2OH	H_2O	CH_2OH
$\parallel$	$\rightarrow$		$\rightarrow$			
CH_2		CH_2Cl		CH_2CN		CH_2
						COOH
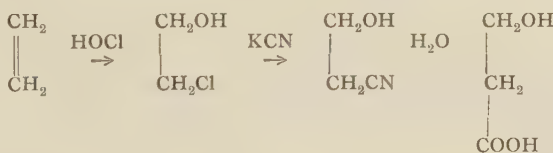
It is formed from acrylic acid (p. 163) by addition of water under the influence of sodium hydrate, hence its name of hydracrylic acid :—

CH_2		CH_2OH
$\parallel$		
CH	$+ H_2O =$	CH_2
COOH		COOH
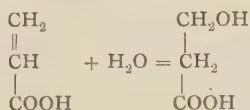
Acrylic acid is formed from β-hydroxypropionic acid by dehydration with strong sulphuric acid.

It is a syrup.

Lactic Acid.

Lactic is formed by the fermentation of sugar by lactic acid bacteria; hence its presence in milk when it turns sour. Lactic acid is contained in muscle, especially after activity, and other organs of the animal body.

Preparation.

The usual method of preparation is by fermentation, i.e. by biological means.

* To a solution of 50 gm. of cane sugar in 500 c.c. of water 20 gm. of chalk, or zinc carbonate, and 20-30 c.c. of sour milk (which contains lactic acid bacteria) are added and the mixture is kept in a warm place, or better in an incubator at 37°, for 6-8 days and occasionally shaken. The chalk, or zinc carbonate, is added to neutralise the lactic acid which hinders the growth of the bacteria. Calcium, or zinc, lactate is formed.

The solution is boiled to kill bacteria, filtered, and evaporated on the water-bath till crystallisation commences and allowed to cool. The lactate is filtered off, pressed between sheets of filter paper, and recrystallised from hot water. The acid is obtained from the salt by decomposition with sulphuric acid, extraction of the liquid with ether, and removal of the ether by distillation.

Too long a time of fermentation must be avoided, as butyric acid may be formed from lactic acid.

Lactic acid is produced from carbohydrates (glucose, fructose, and galactose) by the action of alkali. Methyl glyoxal is formed as an intermediate product and undergoes simultaneous reduction and oxidation :—

$$C_6H_{12}O_6 \rightarrow CH_3 . CO . CHO \rightarrow CH_3 . CHOH . COOH.$$

Lactic acid has been prepared synthetically by several methods, which prove its constitution of a-hydroxypropionic acid.

It is formed (1) by the action of aqueous alkali upon a-chloropropionic acid :—

$$\begin{array}{ccc} CH_3 & & CH_3 \\ | & & | \\ CHCl + NaOH &=& CHOH + NaCl. \\ | & & | \\ COOH & & COOH \end{array}$$
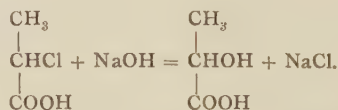
(2) by addition of hydrogen cyanide to acetaldehyde and hydrolysis of the nitrile :—
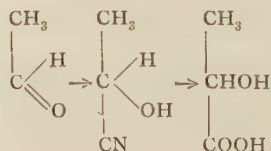
(3) by reduction of pyruvic acid—

$$\begin{array}{cc} CH_3 & CH_3 \\ | & | \\ CO & \rightarrow CHOH \\ | & | \\ COOH & COOH \end{array}$$
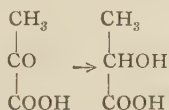
These syntheses show that lactic acid contains the ethylidene radicle $CH_3CH<$. Lactic acid is therefore also known as ethylidene lactic acid.

Properties.

Lactic acid is a syrupy liquid having a sp. gr. of 1·248 at 15°. It is decomposed on distillation at ordinary atmospheric pressure, but at a pressure of ·5-1 mm. it distils at about 85° and then sets to a hygroscopic crystalline solid melting at 18°. It is soluble in water, alcohol, or ether, and is only very slightly volatile with steam.

Lactic acid has the chemical properties of a secondary alcohol and of an acid. The most characteristic salt of lactic acid is the zinc salt. This is prepared by boiling a solution of lactic acid for some time with excess of zinc carbonate and filtering whilst hot. On cooling, zinc lactate crystallises out, if the solution be sufficiently concentrated.

Lactic acid very easily passes into an anhydride, $C_6H_{10}O_5$,

$$2C_3H_6O_3 - H_2O = C_6H_{10}O_5$$

on standing in a dry atmosphere, or on heating to 130-140°. The anhydride is a pale yellow amorphous mass scarcely soluble in water, but passes into lactic acid on boiling with water, or by the action of alkali. This anhydride is probably lactyl-lactic acid :—

$$\begin{array}{c} CH_3 . CHOH . CO—O—CH . COOH. \\ | \\ CH_3 \end{array}$$
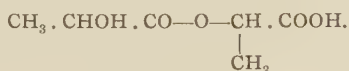
Another compound, lactide, is formed on passing dry air at 150° over lactic acid. This compound appears to be an ester anhydride :—

$$\begin{array}{cc} CH_3 . CHOH—COOH & CH_3 . CH—O—CO \\ = & | \quad \quad | \\ COOH—CHOH - CH_3 & CO—O—CH : CH_3. \end{array}$$

Detection.

(1) Lactic acid in concentrated solution is decomposed by heating with concentrated sulphuric acid with the formation of carbon monoxide ; the gas may be ignited at the mouth of the test tube :—

$$CH_3 . CHOH . COOH = CH_3 . CHO + H_2O + CO.$$
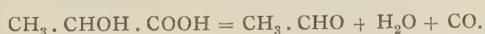
Weak solutions of lactic acid are neutralised with sodium carbonate and evaporated to a small volume before heating with sulphuric acid,

It is oxidised by permanganate in acid solution ; acetaldehyde is formed :—

$$CH_3 . CHOH . COOH + O = CH_3 . CHO + CO_2 + H_2O.$$
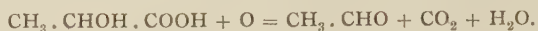
The acetaldehyde can be distilled off during the oxidation, collected and tested, or determined by treatment with excess of standard potassium bisulphite solution, the excess of which is estimated by titration with standard iodine solution.

(2) The formation of acetaldehyde may be detected by *Denigès'* method. The dilute solution of lactic acid is heated for 2 minutes in a boiling water-bath with 10 times its volume of concentrated sulphuric acid. The liquid is cooled, and 2 or 3 drops of a 5 per cent. solution of guaiacol in alcohol are added. On mixing, a rose-red colour—formed from the aldehyde and guaiacol—is produced which increases in intensity on standing.

* (3) Lactic acid forms a soluble deep yellow ferric salt. If a dilute solution of ferric chloride (scarcely coloured) be treated with a few drops of a dilute solution of lactic acid, the colour becomes yellow.

Other hydroxy acids and oxalic acid also give a similar colour.

* (4) *Uffelmann's Test.*—Uffelmann's reagent—a 1 or 2 per cent. solution of phenol treated with ferric chloride solution till of a distinctly violet colour—is changed to yellow on the addition of lactic acid.

Note.—Mineral acids decolorise the reagent ; other organic acids also give a yellow or brownish colour.

* (5) Lactic acid gives the iodoform reaction (p. 63).

(6) *Thiophene Test.*—A few drops of a 1 per cent. solution of lactic acid in alcohol are added to 5 c.c. of concentrated sulphuric acid containing 3 drops of saturated copper sulphate solution and heated in a boiling water-bath for 5 minutes. Two drops of a ·2 per cent. alcoholic solution of thiophene are added to the cooled solution, and on again warming a cherry-red colour is formed (*Hopkins*).

These tests for lactic acid are not easily observed in extracts of organs, etc., which contain lactic acid. The lactic acid should be extracted with ether, the ethereal solution evaporated and the residue then tested for lactic acid.

Stereoisomerism.

Lactic acid is the simplest compound which exhibits the phenomenon of circular polarisation. Most natural compounds exhibit this phenomenon. Circular polarisation is the property of rotating a ray of polarised light to either the right or the left (see p. 284).

According to its source lactic acid may be either dextrorotatory, or laevorotatory, or inactive. Lactic acid from muscle—sarcolactic acid

—is dextrorotatory.   Certain bacteria produce laevorotatory lactic acid. Ordinary fermentation lactic acid is inactive.

The examination of natural substances which exhibit circular polarisation has shown that they all contain one or more *asymmetric* carbon atoms, i.e. carbon atoms combined with four different groups. The usual representation of a carbon atom shows its four valencies and attached radicles arranged round it in one plane.   An isomerism then occurs if any two radicles differ from the other two, thus

$$\begin{array}{ccc} & H & & & H \\ & | & & & | \\ H- & C & -Cl & \text{and} & Cl- & C & -Cl \\ & | & & & | \\ & Cl & & & H \end{array}$$
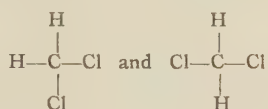
are two different representations of dichloromethane, but only one dichloromethane is known.

By representing the carbon atom as a regular tetrahedron and placing the different groups at the four apices, Van't Hoff and Le Bel have given an explanation of the above difference and of the existence of the three different lactic acids.   On the basis of a regular tetrahedron, the four bonds and attached radicles do not lie in one plane, but are arranged in three dimensions of space.   If models of the two possible
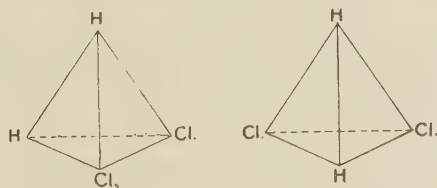
dichloromethanes be made, it is easily seen, by rotating one of the models, that they are identical.

A real difference only occurs when the four radicles attached to the carbon atom are different.   Two different models can now be constructed, one of which is the mirror image of the other, thus in the case of lactic acid
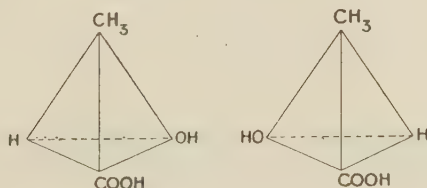
These models on rotation can never be made to coincide.   Adopting any arrangement of the groups round the tetrahedron, e.g. H, CH_3, OH,

the reverse arrangement HO, CH_3, H is shown by the mirror-image. The order of the groups is clockwise in the first model, and counterclockwise in the second model. If therefore one variety is represented by the first arrangement, the mirror-image of the figure represents the opposite variety. Projected on to a plane surface the following formulæ are then obtained :—

$$
\begin{array}{cc}
CH_3 & CH_3 \\
| & | \\
HO.C.H & H.C.OH \\
| & | \\
COOH & COOH.
\end{array}
$$
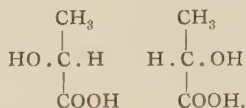
One figure will represent laevo or *l*-lactic acid, the other figure dextro or *d*-lactic acid. *d*-lactic acid and *l*-lactic acid rotate polarised light to the same degree, but in opposite directions. A mixture of the two in equal quantities represents inactive or *dl*-lactic acid. This is proved by the separation of inactive lactic acid into its constituent *d*- and *l*-forms by the fractional crystallisation of its strychnine salt.

These optical isomers are known as stereoisomers and the phenomenon as stereoisomerism. (See further p. 152.)

If a compound with an asymmetric carbon atom be synthesised, the optically inactive mixture is obtained, because the possibility of the formation of one arrangement is as likely as that of the other. The *d*- and *l*- constituents can be separated from this mixture by fractional crystallisation of salts with optically active compounds and by other means (see p. 153). The direct preparation of an optically active compound has only been effected in a few cases.

The optical difference of these two isomers depends upon a different arrangement of the atoms inside the molecule and not upon a different arrangement of the molecules themselves. These substances with an asymmetric carbon atom show their optical properties in solution ; in certain cases amongst the terpenes and camphors the optical properties are observed also in the gaseous state if they can be volatilised without decomposition. Other optically active compounds, such as quartz and sodium chlorate crystals only show the property in the solid condition ; their solutions are optically inactive. The activity of quartz and sodium chlorate depends upon the arrangement of the molecules themselves.

Pyruvic Acid, or Ketopropionic Acid.

The work of recent years shows that pyruvic acid and other ketonic acids are very probably intermediate products in the metabolism of fatty acids and amino acids. Pyruvic acid appears to be a stage in

the transformation of sugar into alcohol and carbon dioxide and into lactic acid :—

$$\begin{array}{cccc} CH_3 & CH_3 & CH_3 & CH_3 \\ | & | & | & | \\ CHOH \leftarrow & CO & \rightarrow CHO \rightarrow & CH_2OH \\ | & | & + & \\ COOH & COOH & CO_2. & \end{array}$$
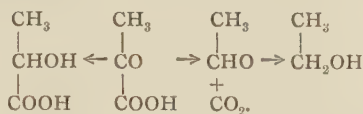
Preparation.

Pyruvic acid is usually prepared from tartaric acid.   A mixture of 500 gm. of tartaric acid and 780 gm. of potassium bisulphate is distilled from a 2 li. copper retort provided with a condenser; the receiver is cooled with ice. The distillate is redistilled *in vacuo* using a fractionating column.   A yield of about 60 per cent. is obtained.   A complicated reaction occurs, involving loss of carbon dioxide and water from the tartaric acid.

Properties.

Pyruvic acid is a liquid which freezes at 9° and boils at 168° under atmospheric pressure, or at 50-60° at 12 mm. pressure.   It smells very like acetic acid and is soluble in water.   It has the properties of an acid and of a ketone and forms a characteristic hydrazone.

Test.

Hurtley has described the following delicate test for pyruvic acid.   The test depends upon the formation of a red colour on oxidising the phenyl-hydrazone of pyruvic acid.   The reaction is positive at a dilution of 1 in 10,000 and can be obtained at a dilution of 1 in 100,000.

10 c.c. of pyruvic acid (1 per cent.) are treated with 10 c.c. of phenyl-hydrazine hydrochloride solution (6·41 gm. in 500 c.c. acid = 5 gm. pyruvic acid) ; about 2 c.c. of persulphuric acid (25 gm. $K_2S_2O_8$ are ground up with 50 gm. H_2SO_4 and left for 1 hour, then poured upon ice and diluted to 500 c.c.) are added avoiding an excess and then 5 c.c. concentrated hydrochloric acid.   A red colour develops.

Malonic Acid.

This acid is found in beetroot as its calcium salt.   It was originally prepared by the oxidation of malic acid with potassium bichromate and sulphuric acid, but is usually made by the cyanide synthesis from chloracetic acid :—

$$\begin{array}{ccc} CH_2Cl & CH_2.CN & CH_2.COOH \\ | & \rightarrow \quad | & \rightarrow \quad | \\ COOH & COOH & COOH. \end{array}$$

Potassium chloracetate is boiled with potassium cyanide; the product is hydrolysed with hydrochloric acid and the solution evaporated to dryness. The residue is extracted with ether, and the malonic acid obtained by distillation of the ether.

Malonic acid is a colourless, crystalline solid which melts at 132°, and is readily soluble in water, alcohol, and ether.   On heating

to 140-150°, it loses carbon dioxide and is converted into acetic acid :—

$$
\begin{array}{l}
\text{COOH} \\
|\\
\text{CH}_2 \quad = \text{CO}_2 + \quad
\begin{array}{l}
\text{CH}_3 \\
|\\
\text{COOH.}
\end{array} \\
|\\
\text{COOH}
\end{array}
$$
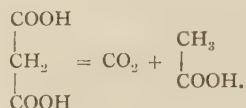
This reaction is given by all acids in which two carboxyl groups are attached to the same carbon atom.

Malonic acid, in the form of its diethyl ester, is much used in organic synthesis. The essential property of malonic ester is the formation of sodium derivatives by the action of metallic sodium, and the replacement of the sodium by alkyl groups. This reaction is given by other compounds containing a —CH_2— group between two CO groups (see also aceto-acetic ester). The method of synthesis is seen from the following reactions :—

$$
\begin{array}{ccccc}
\begin{array}{l}\text{COOC}_2\text{H}_5\\|\\\text{CH}_2\\|\\\text{COOC}_2\text{H}_5\end{array}
&\xrightarrow{\text{Na}}&
\begin{array}{l}\text{COOC}_2\text{H}_5\\|\\\text{CHNa}\\|\\\text{COOC}_2\text{H}_5\end{array}
&\xrightarrow{\text{C}_2\text{H}_5\text{I}}&
\begin{array}{l}\text{COOC}_2\text{H}_5\\|\\\text{CH}.\text{C}_2\text{H}_5\\|\\\text{COOC}_2\text{H}_5\end{array}
\xrightarrow{\text{H}_2\text{O}}
\begin{array}{l}\text{COOH}\\|\\\text{CH}.\text{C}_2\text{H}_5\\|\\\text{COOH}\end{array}
\rightarrow
\begin{array}{l}\text{CO}_2+\\\\\text{CH}_2\text{C}_2\text{H}_5\\|\\\text{COOH}\end{array}
\end{array}
$$

| Malonic ester. | Sodio malonic ester. | Ethyl malonic ester. | Ethyl malonic acid. | Butyric acid. |

$$
\begin{array}{ccccc}
\text{C}\!\!\begin{array}{l}{}^{\text{H}}\\{}_{\text{C}_2\text{H}_5}\end{array}\!\!
\begin{array}{l}\text{COOC}_2\text{H}_5\\\\\\\text{COOC}_2\text{H}_5\end{array}
&\xrightarrow{\text{Na}}&
\text{C}\!\!\begin{array}{l}{}^{\text{Na}}\\{}_{\text{C}_2\text{H}_5}\end{array}
&\xrightarrow{\text{C}_2\text{H}_5\text{I}}&
\text{C}\!\!\begin{array}{l}{}^{\text{C}_2\text{H}_5}\\{}_{\text{C}_2\text{H}_5}\end{array}
\xrightarrow{\text{H}_2\text{O}}
\text{C}\!\!\begin{array}{l}{}^{\text{C}_2\text{H}_5}\\{}_{\text{C}_2\text{H}_5}\end{array}
\rightarrow
\text{HC}\!\!\begin{array}{l}{}^{\text{C}_2\text{H}_5}\\{}_{\text{C}_2\text{H}_5}\end{array}
\end{array}
$$

| Ethyl malonic ester. | Sodio-ethyl malonic ester. | Diethyl malonic ester. | Diethyl malonic acid. | Diethyl acetic acid. |

Diethyl malonic ester is prepared directly by passing hydrochloric acid gas into cyanacetic acid dissolved in alcohol.

COMPOUNDS CONTAINING FOUR CARBON ATOMS.

The vegetable acids, malic and tartaric, and the two acids, β-hydroxybutyric and acetylacetic, which are found in urine in cases of diabetes are the chief biological representatives of this group of compounds. Succinic acid is the dibasic acid of this series; malic acid is monohydroxy-succinic acid; tartaric acid is dihydroxy-succinic acid :—

COOH	COOH	COOH	CH_3	CH_3
CH_2	CHOH	CHOH	CHOH	CO
CH_2	CH_2	CHOH	CH_2	CH_2
COOH	COOH	COOH	COOH	COOH
Succinic acid.	Malic acid.	Tartaric acid.	β-hydroxy-butyric acid.	Acetyl-acetic acid.
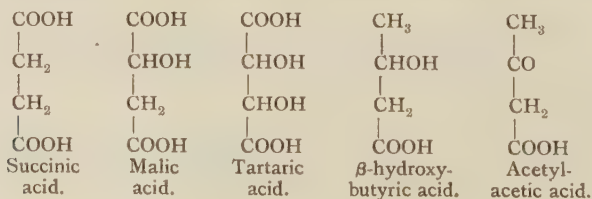
Succinic Acid.

Succinic acid occurs in certain lignites and fossils, in lettuce, unripe grapes, and other fruits. It has also been found in animal tissues and meat extracts.

Succinic acid is obtained by the dry distillation of amber. The aqueous distillate is filtered whilst hot to separate oil, and on cooling crystals of succinic acid are deposited. They may be purified by boiling with nitric acid and recrystallisation.

Succinic acid is usually prepared by the fermentation by yeast of calcium malate, or ammonium tartrate. It is formed by the reduction of these compounds. Reduction of malic acid and tartaric acid with hydriodic acid yields succinic acid.

The constitution of succinic acid is proved by its synthesis :—

$$\begin{matrix} CH_2 \\ \| \\ CH_2 \end{matrix} \rightarrow \begin{matrix} CH_2Br \\ | \\ CH_2Br \end{matrix} \rightarrow \begin{matrix} CH_2 . CN \\ | \\ CH_2 . CN \end{matrix} \rightarrow \begin{matrix} CH_2 . COOH \\ | \\ CH_2 . COOH. \end{matrix}$$
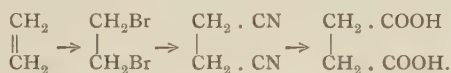
Succinic acid crystallises in colourless prisms, or plates, which melt at 182°. It is not readily soluble in cold water, but dissolves readily in alcohol and sparingly in ether. It is insoluble in chloroform and benzene.

On heating, it emits suffocating fumes ; at higher temperatures it boils and gives a sublimate of succinic anhydride :—

$$\begin{matrix} CH_2 . COOH \\ | \\ CH_2 . COOH \end{matrix} = H_2O + \begin{matrix} CH_2 . CO \\ | \\ CH_2 . CO \end{matrix}\Big\rangle O.$$
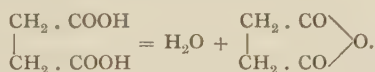
A compound containing a ring of atoms is thus formed.

Ammonium succinate, and succinamide, on heating give succinimide, another compound with a ring of atoms :—

$$\begin{matrix} CH_2 . COONH_4 \\ | \\ CH_2 . COONH_4 \end{matrix} = NH_3 + 2H_2O + \begin{matrix} CH_2 . CO \\ | \\ CH_2 . CO \end{matrix}\Big\rangle NH$$

Succinimide.
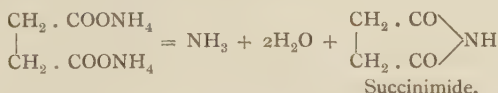
Malic Acid.

Malic acid is contained in gooseberries, unripe apples, pears, and other fruits. It is usually prepared from rhubarb stalks, or unripe mountain ash berries. The juice is boiled with milk of lime ; the neutral calcium salt is precipitated. The salt is recrystallised from dilute nitric acid and the acid salt is so obtained. It is decomposed with the calculated quantity of sulphuric acid, the liquid is filtered from calcium sulphate and evaporated. Malic acid crystallises out.

The constitution of malic acid is proved by its reduction with

hydriodic acid to succinic acid, and by its preparation from monobromo-succinic acid by the action of aqueous alkali :—

$$\begin{array}{c} CH_2 . COOH \\ | \\ CHBr . COOH \end{array} \rightarrow \begin{array}{c} CH_2 . COOH \\ | \\ CHOH . COOH. \end{array}$$
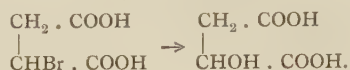
Malic acid crystallises in groups of colourless 4 or 6-sided prisms. It is deliquescent and readily soluble in water, alcohol, and ether.

On heating to about 180°, it melts and loses water yielding fumaric and maleic acids.

Malic acid is optically active and contains one asymmetric carbon atom. The natural form is *l*-malic acid.

The salts of malic acid resemble those of citric, oxalic, and tartaric acids. Calcium malate is not precipitated in the cold. On boiling in neutral and concentrated solution, calcium malate is precipitated; alcohol precipitates calcium malate from dilute aqueous solution. A mixture of oxalic, tartaric, citric, and malic acids may thus be separated. The oxalate and tartrate are precipitated from dilute solution in the cold ; on boiling the filtrate, calcium citrate is precipitated, and on adding 2 volumes of alcohol to the filtrate, calcium malate is thrown down.

Tartaric Acid.

Tartaric acid occurs in certain plant juices ; its only important source is grape juice. During fermentation a deposit forms on the bottom—lees—and a crystalline crust on the sides—tartar or argol—of the cask. The argol consists mainly of potassium hydrogen tartrate with a small amount of calcium tartrate. Their precipitation is due to their insolubility in the alcohol as it is produced. If the crude argol be boiled with water and filtered and the solution crystallised, cream of tartar separates out, the term cream of tartar having arisen from the fact that the salt collects in crusts on the surface during the evaporation.

Preparation.

Tartaric acid is prepared from tartar by dissolving it in water and neutralising with lime. Insoluble calcium tartrate is thrown down and from the solution, which still contains tartaric acid, insoluble calcium tartrate is precipitated by adding calcium sulphate, or calcium chloride. The insoluble calcium salt is decomposed with sulphuric acid and tartaric acid isolated from the solution by crystallisation.

Properties.

Tartaric acid crystallises in large hemihedral monoclinic prisms which are colourless and transparent. It melts at 167·170° and is easily soluble in water and alcohol, but insoluble in ether.

Tartaric acid is optically active ; the ordinary tartaric acid is dextro-rotatory having $[a]_D = 13\cdot1°$ for a 15 per cent. solution and $= 14\cdot7°$ for a 2 per cent. solution.   A laevo-tartaric acid and two inactive forms of tartaric acid also exist.

Tartaric acid behaves as a dibasic acid.   Of the numerous salts of tartaric acid, the sodium potassium salt or Rochelle salt, acid potassium tartrate, and tartar emetic are of chief importance :—

$$
\begin{array}{lll}
\text{CHOH . COONa} & \text{CHOH . COOH} & \text{CHOH . COOK} \\
| & | & | \\
\text{CHOH . COOK} & \text{CHOH . COOK} & \text{CHOH . COO(SbO)} \\
\text{Rochelle salt.} & \text{Acid potassium} & \text{Tartar emetic.} \\
& \text{tartrate.} &
\end{array}
$$
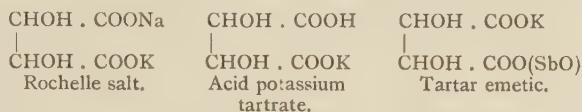
Constitution.

On reduction with hydriodic acid, it is converted firstly into malic acid and then into succinic acid.   Its constitution is shown by its synthesis from

(1) dibromosuccinic acid :—

$$
\begin{array}{l}
\text{CHBr . COOH} \\
| \qquad\qquad\; + 2\text{KOH} = \\
\text{CHBr . COOH}
\end{array}
\begin{array}{l}
\text{CHOH . COOH} \\
| \qquad\qquad\; + 2\text{KBr.} \\
\text{CHOH . COOH}
\end{array}
$$

(2) glyoxal :—

$$
\begin{array}{l}
\text{CHO} \\
| \quad \rightarrow \\
\text{CHO}
\end{array}
\begin{array}{l}
\text{CHOH . CN} \\
| \qquad\quad \rightarrow \\
\text{CHOH . CN}
\end{array}
\begin{array}{l}
\text{CHOH . COOH} \\
| \\
\text{CHOH . COOH.}
\end{array}
$$
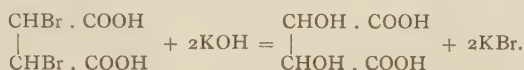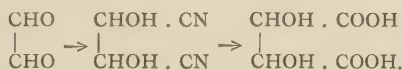
The formation of tartaric acid from fumaric and maleic acids (p. 164) is also of interest.   Fumaric acid on oxidation gives racemic acid, maleic acid gives meso-tartaric acid.

Reactions.

* (1) On heating, tartaric acid melts and chars giving off an odour resembling that of burnt sugar.

* (2) Tartaric acid chars almost immediately when it is heated with concentrated sulphuric acid.

* (3) A white precipitate of silver tartrate is formed on adding silver nitrate to a neutral solution of a tartrate.   The precipitate dissolves in ammonia and when this solution is slowly warmed a silver mirror is formed on the sides of the vessel.

* (4) On adding calcium chloride to a *cold* solution of a neutral tartrate (sodium potassium tartrate), a white precipitate of calcium tartrate is formed.   This precipitate, after filtering, and washing, is soluble in acetic acid and caustic soda (free from carbonate) ; on boiling the solution in the latter, it is reprecipitated (distinction from calcium oxalate).

*   (5) A precipitate of potassium hydrogen tartrate is formed on adding potassium chloride and acetic acid to a not too dilute solution of a tartrate.

This reaction is used in estimating tartaric acid.

*   (6) The presence of tartaric acid, or tartrates, in a solution prevents the precipitation of metallic hydroxides by caustic soda.

*   (a) No precipitate is formed if caustic soda be added to ferric chloride solution containing some tartrate solution, but a yellow-brown solution results.

*   (b) A dark-blue solution results, if caustic soda be added to copper sulphate solution containing a tartrate.

This property is used in the preparation of Fehling's solution.

Stereoisomerism (contd.).

Tartaric acid contains two asymmetric carbon atoms, each of them with the same four radicles :—

$$
\begin{array}{ll}
\text{COOH} & \text{CHOH . COOH} \\
| & | \\
\text{H—C—OH} & \text{H—C—OH} \\
| & | \\
\text{CHOH . COOH} & \text{COOH.}
\end{array}
$$
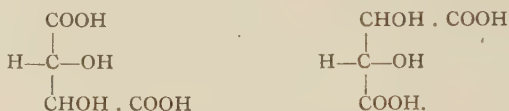
Four different modifications are therefore possible.   These modifications become clear on reference to the models :—
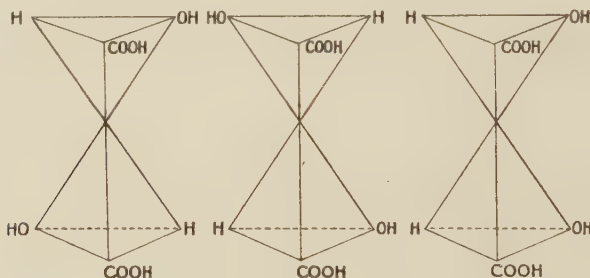
Projected on to a plane surface, they appear as

$$
\begin{array}{ccc}
\text{COOH} & \text{COOH} & \text{COOH} \\
| & | & | \\
\text{H—C—OH} & \text{HO—C—H} & \text{H—C—OH} \\
| & | & | \\
\text{HO—C—H} & \text{H—C—OH} & \text{H—C—OH} \\
| & | & | \\
\text{COOH} & \text{COOH} & \text{COOH} \\
d\text{-tartaric acid.} & l\text{-tartaric acid.} & \text{Mesotartaric acid.}
\end{array}
$$
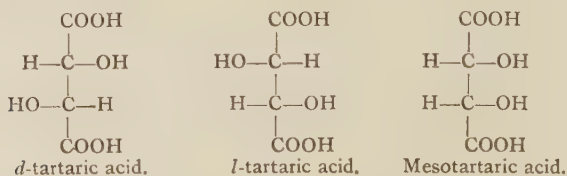
It is necessary to consider each asymmetric carbon atom and the order of the groups looking at the models from the centre point. Adopting the order of H, OH, COOH, the first model shows the

arrangement round each carbon atom to be the same.   Both are rotat-
ing to the right and cause dextro rotation.   In the second model, the
arrangement of the groups is again the same, but counter-clockwise.
Both are rotating to the left and cause laevo rotation.   A mixture of
these forms in equal quantities will give an inactive tartaric acid, or
racemic acid, capable of separation.

In the third model, the groups around one of the carbon atoms are
clockwise, but around the other carbon atom counter-clockwise.   The
rotations of the two are the same but in opposite directions, and they
neutralise one another by internal compensation.   This inactive acid
cannot be separated.   It is known as meso-tartaric acid.

Separation of Optically Active Tartaric Acids.

The separation of the mixture of d- and l-tartaric acids, such as is
found in grapes, together with d-tartaric acid was first effected by
Pasteur who discovered three methods, which can be used also in the case
of other similar optically active mixtures.

1. It was observed that sodium ammonium tartrate crystallised in
two shapes differing in the position of their hemihedrally formed
facets.   The one kind was the mirror image of the other :—
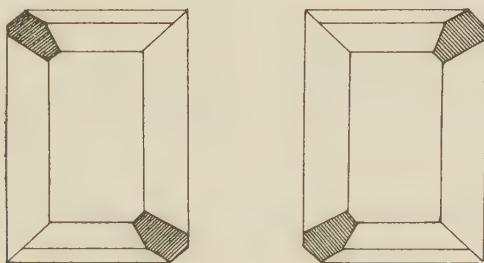
By mechanically picking out these different crystals and preparing from
them the free acid, Pasteur obtained from the one kind laevo-tartaric
acid, and from the other kind dextro-tartaric acid.

2. Salts of the optically active acids with alkaloids (quinine,
strychnine, etc.) were found to have different solubilities.   A mixture of
the salts of the mixed optically active acid could be separated by
fractional crystallisation.   In the case of the mixed optically active
tartaric acids, the cinchonine salt was the most useful.   (See lactic
acid.)

This method is of the most general application.   It can be used in
the converse way to separate a mixture of optically active bases by
making a salt with, for example, d-tartaric acid.

3. Certain moulds, e.g. *Penicillium glaucum* (also yeasts and bacteria), if grown or cultivated upon a solution of the salt of the acid, decompose one isomer, but not the other.   In the case of tartaric acid, the dextro form is destroyed.

The three methods of separating the optically active tartaric acids from inactive racemic acid, discovered by Pasteur, may be called :—

1. *Mechanical.*
2. *Chemical.*
3. *Biological.*

These methods are used for separating other mixtures of optically active substances.   The first method is only occasionally available, as such distinct crystalline forms are rare.   The third method gives only one optically active form.   The second method is the principal one. Many other alkaloids can be used.   This method is used in a converse way for separating optically active bases by making salts with optically active acids, and fractional crystallisation.

The internally compensated tartaric acid cannot be separated by any of these methods.   The internally compensated form is known as the meso-form.

The mixture of optically active tartaric acids and other similar mixtures capable of separation is generally referred to as the racemic tartaric acid, or racemic mixture.

The optically active forms of tartaric acids are found in nature. By prolonged heating of dextrorotatory tartaric acid to 165° with a small quantity of water, or boiling with alkali, it is changed to meso-tartaric acid.

Synthetically prepared tartaric acid is a mixture of *d*- and *l*-tartaric acids.   By using the separation with the optically active base it can be obtained in its optically active forms.   The complete synthesis of a natural optically active compound can thus be effected.   The method of formation of the single optically active compound by the living organism is not yet known.   In only a few cases has the direct preparation of an optically active compound been effected, and no insight is given of the mechanism in nature.

Citric Acid.

Citric acid is another hydroxy acid, which occurs in the free state in the juices of many plants.   Small quantities are present in milk.

Preparation.

About 5·5 per cent. of citric acid is obtainable from good lemons ; about 1 per cent. from unripe gooseberries.   It is usually extracted

from lemons, limes, and bergamot. The hot liquid is neutralised with calcium carbonate, and the calcium citrate so obtained is decomposed by sulphuric acid in equivalent amount. The solution on evaporation gives citric acid.

Citric acid is β-hydroxy-tricarballylic acid, a tribasic acid. Its formula is proved by its synthesis :—

$$\begin{array}{c}
CH_2OH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\xrightarrow{HCl}
\begin{array}{c}
CH_2Cl \\
| \\
CHOH \\
| \\
CH_2Cl
\end{array}
\xrightarrow{O}
\begin{array}{c}
CH_2Cl \\
| \\
CO \\
| \\
CH_2Cl
\end{array}
\xrightarrow{HCN}
\begin{array}{c}
CH_2Cl \\
| \\
C{<}^{OH}_{CN} \\
| \\
CH_2Cl
\end{array}
\xrightarrow{H_2O}
\begin{array}{c}
CH_2Cl \\
| \\
C{<}^{OH}_{COOH} \\
| \\
CH_2Cl
\end{array}
\xrightarrow{KCN}
\begin{array}{c}
CH_2CN \\
| \\
C{<}^{OH}_{COOH} \\
| \\
CH_2CN
\end{array}
\to
\begin{array}{c}
CH_2 . COOH \\
| \\
C(OH) . COOH \\
| \\
CH_2 . COOH
\end{array}$$
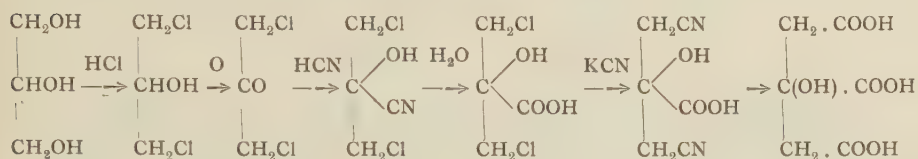
It closely resembles tartaric acid, but there are many points of difference.

Properties.

Citric acid is obtainable either as a crystalline powder, or in transparent colourless prisms having the formula $C_6H_8O_7 . H_2O$. It has a strong acid taste, is very easily soluble in water, and is also soluble in dilute and absolute alcohol ; it is almost insoluble in ether, chloroform, petroleum ether, and benzene.

Reactions.

* (1) On heating, citric acid loses water becoming anhydrous, melts and decomposes, giving off acid fumes of aconitic acid.

* (2) When heated with concentrated sulphuric acid, citric acid chars slowly.

* (3) Silver citrate is precipitated on adding silver nitrate to a neutral solution of citric acid, or a citrate. The precipitate dissolves in ammonia, but the solution on warming is not reduced and does not form a silver mirror.

* (4) Calcium citrate is not precipitated when calcium chloride is added to a cold neutral solution of a citrate. This salt is less soluble in hot water and hence, on boiling the solution, calcium citrate is precipitated ; it dissolves again as the solution cools.

* (5) Citric acid does not form an insoluble acid potassium salt when its solutions are treated with potassium chloride and acetic acid.

Aceto-Acetic Ester.

Ethyl aceto-acetate was prepared in 1863 by Geuther by acting upon ethyl acetate with sodium and acidifying the product with acetic acid. An oil resulted ; it was separated and purified by distillation :—

$$CH_3 . COOC_2H_5 + CH_3 . COOC_2H_5 = CH_3 . CO . CH_2 . COOC_2H_5 + HOC_2H_5.$$
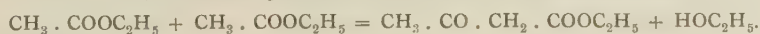
Ethyl aceto-acetate is a liquid with a fruity smell which boils at 182°.

Aceto-acetic ester exists in two tautomeric, or desmotropic, modifications, a keto-form and an enol form :—

$$
\begin{array}{ccc}
\text{CH}_3 & & \text{CH}_3 \\
| & & | \\
\text{CO} & \longrightarrow & \text{C . OH} \\
| & & \| \\
\text{CH}_2 & \longleftarrow & \text{CH} \\
| & & | \\
\text{COOC}_2\text{H}_5 & & \text{COOC}_2\text{H}_5 \\
\text{Keto form.} & & \text{Enol form.}
\end{array}
$$
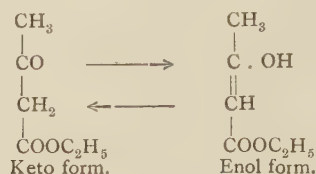
The ordinary ester consists of about 90 per cent. keto form and 10 per cent. enol form. The keto form changes comparatively slowly into the enol form, the enol form comparatively rapidly into the keto form. The formation of a sodium derivative and the ferric chloride reaction are due to the -enol form.

Aceto-acetic ester is largely used in organic synthesis like malonic ester, thus :—
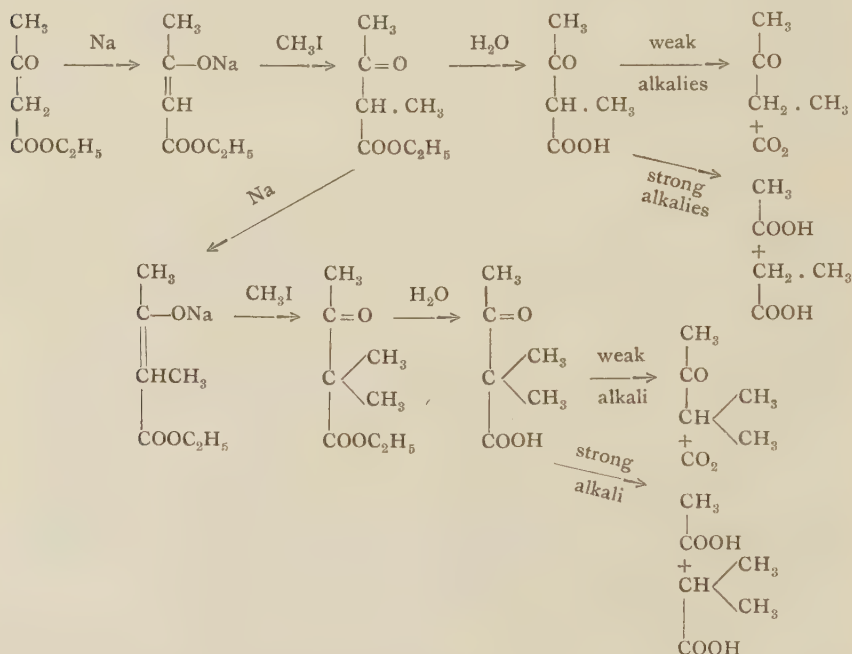
According to the final stage, either a ketone and CO_2 (ketone hydrolysis), or acetic acid and another acid (acid hydrolysis) are produced.

Aceto-Acetic Acid, or Acetylacetic Acid.

Aceto-acetic acid can be prepared from the ester by hydrolysis. It is a very unstable, strongly acid, hygroscopic syrup which decomposes

on warming into acetone and carbon dioxide.   Solutions of aceto-acetic acid are readily decomposed on distilling with dilute acid or alkali.

Traces of aceto-acetic acid occur in normal urine—2-4 mg. in 24 hours.   In diabetes large amounts of aceto-acetic acid may be present. The amount is increased in starvation, on a diet of protein, and on a diet of fat—i.e. whenever there is a shortage of carbohydrate.   The excretion of aceto-acetic acid is lessened, if carbohydrate be added to the food.

The formation of aceto-acetic acid in the organism from fat when carbohydrate is withheld from the food explains its formation in diabetes. Here the organism has lost its power of utilising the carbohydrate in the food, or its power of utilising carbohydrate is greatly diminished.

The origin of the aceto-acetic acid in the urine appears to be partly from the protein of the food, but mainly from the fat.   The work of Knoop and of Dakin has shown that the oxidation of the fatty acids takes place at the β-carbon atom ; the long chains are broken down with the loss of 2 carbon atoms at a time.   This accounts in part for the occurrence of those fatty acids in nature containing an even number of carbon atoms.   Butyric acid, if present as such, or formed by the oxidation of higher fatty acids by β-oxidation, is oxidised and converted into aceto-acetic acid.

Aceto-acetic acid is then converted by reduction into β-hydroxybutyric acid, and *vice versa*, β-hydroxybutyric acid can be converted by oxidation into aceto-acetic acid.   It seems most likely that aceto-acetic acid, the keto acid, is the chief product of the oxidation of butyric acid :—

$$CH_3 . CH_2 . CH_2 . COOH$$

$$CH_3 . CO . CH_2 . COOH \rightleftarrows CH_3 . CHOH . CH_2 . COOH.$$
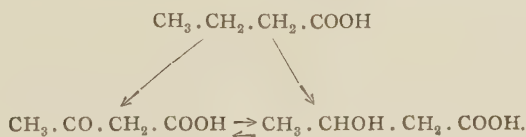
Aceto-acetic acid is very unstable and is readily converted into acetone and carbon dioxide.   This decomposition occurs spontaneously in normal urine.

The three closely related substances, β-hydroxybutyric acid, aceto-acetic acid, and acetone are generally referred to in medical literature as the acetone bodies.   There is no basis for the older statement that mild cases of diabetes excrete only acetone, that severer cases excrete acetone and aceto-acetic acid, and that still severer cases excrete β-hydroxybutyric acid in addition.   It seems to have been due to a misinterpretation of the tests and to the inadequacy of our methods of estimating these substances.

The occurrence of acetone in the breath of diabetics can be accounted for by the difference in the blood circulation.   This is slow through the systemic system and lung capillaries through which the blood passes before it goes to the kidney.   Venous blood is more acid than arterial blood, so that the conditions for the decomposition of aceto-acetic acid are most favourable.   Acetone is very volatile, and if decomposition occurs during the passage of the blood through the lungs it would pass into the expired air.[1]

Tests.

The various tests for aceto-acetic acid were reviewed and summarised by W. H. Hurtley in 1913,[2] and the following preparation of a dilute solution of sodium aceto-acetate, conveniently used for the tests, was described :—

13 gm. of pure ethyl aceto-acetate are treated with 100 c.c. of normal caustic soda and diluted to 500 c.c.   The ester is almost completely hydrolysed by allowing this solution to stand for 44·5 hours. A solution containing 1 gm. of aceto-acetic acid is obtained by diluting it to 5000 c.c., or better, by diluting 49·2 c.c. of this solution to 1 litre.

(1) *Gerhardt's Ferric Chloride Test.*—Dilute ferric chloride solution, added drop by drop, to about 10 c.c. of the solution gives a claret-red colour.

The delicacy of this reaction is very nearly 1 in 100,000.

In applying this test to urine, a precipitate of ferric phosphate is formed.   Ferric chloride is added so long as a precipitate is produced and the ferric phosphate filtered off.   On adding ferric chloride to the filtrate, the claret colour appears if aceto-acetic acid be present.

The delicacy of the reaction with urine is less than given above on account of the presence of other substances which give a red colour with the iron salt.

There is also the disadvantage in testing urine that aromatic compounds—salicylates, antipyrine—which are excreted after their administration, also give a violet colour with ferric chloride.

To avoid confusion the urine is shaken with benzene, or chloroform, which removes salicylic acid.   The urine is then acidified with sulphuric acid and shaken with ether which extracts the aceto-acetic acid.   The ethereal solution on shaking with dilute ferric chloride solution will give the claret colour.   The colour disappears on standing for 12-24 hours.

---

[1] See the excellent account by Kennaway in the "Guy's Hospital Reports," Vol. LXVII.

[2] "Lancet" for 26th April.

Thiocyanic acid is also extracted by ether, but the colour of ferric thio-cyanate is permanent.

The statement that the colour if produced by aceto-acetic acid dis-appears on boiling is, according to Kennaway, not exactly true.   The colour in either case becomes paler and redder, and in the case of aceto-acetic acid a reddish flocculent precipitate appears on boiling.

*   (2) *Legal's Sodium Nitroprusside Test.*—On adding 3 drops of a freshly prepared 5 per cent. solution of sodium nitroprusside to about 10 c.c. of the solution and rendering alkaline with a few drops of caustic soda, a deep red colour is formed.   The colour changes to magenta on acidifying with acetic acid.

When applied to urine it should be remembered that creatinine gives a similar colour reaction.

*   (3) *Rothera's Sodium Nitroprusside Test.*—10 c.c. of the solution are saturated with ammonium sulphate by adding 5 gm. of the crystals ; 3 drops of 5 per cent. sodium nitroprusside and 2 c.c. of strong ammonia are then added.   A fine permanganate colour is produced.

1 part of aceto-acetic acid in 100,000 gives the reaction in 2 minutes, 1 part in 400,000 in 5 minutes.

This reaction was described as characteristic for acetone, but was shown by Hurtley to be far more delicate for aceto-acetic acid.

β Hydroxybutyric Acid.

β-Hydroxybutyric acid is readily prepared by the reduction of aceto-acetic acid.   In this way it probably arises in the animal body. β-hydroxybutyric acid contains an asymmetric carbon atom and can therefore occur in three forms—dextro, laevo, and inactive.   The laevo form is present in urine in diabetes and under the conditions detailed under aceto-acetic acid.   The dextro form is produced by the reduction of aceto-acetic acid by yeast.   The inactive form is pro-duced by chemical reducing agents, such as sodium amalgam.

β-hydroxybutyric acid is usually obtained as a colourless syrup, but has been prepared in colourless crystalline plates by Magnus Levy ; these sinter at 47-48·5° and melt at 49-50°.   It is very hygro-scopic.   It is easily soluble in water, alcohol, ether, ethyl acetate, and acetone, but not in benzene and petroleum ether.

The rotation of *l*-β-hydroxybutyric acid is $[a]_D^{20} = -24\cdot12°$ at temperatures between 17 and 22° and in concentration less than 12 per cent.

Several salts have been prepared in a crystalline condition, e.g. silver, calcium, zinc.

Detection.

β-hydroxybutyric acid has no reactions like aceto-acetic acid. Its detection is therefore indirect.

(1) It is converted into α-crotonic acid by loss of water either by heating alone, or more readily by heating with dilute sulphuric acid :—

$$CH_3 . CHOH . CH_2 . COOH = CH_3 . CH{=}CH . COOH + H_2O.$$

(2) By oxidation with potassium bichromate and sulphuric acid, or with hydrogen peroxide and ferrous sulphate, it is converted into aceto-acetic acid and then into acetone by decomposition of the aceto-acetic acid.

The detection of hydroxybutyric acid in urine is carried out most certainly by isolation of the substance by extracting with ether.

Levulinic Acid, $CH_3 . CO . CH_2 . CH_2 . COOH.$

Levulinic acid, γ-keto-valerianic acid, or acetyl propionic acid, is formed on heating hexoses (p. 258), especially fructose, with dilute hydrochloric acid, and consequently is sometimes made use of for detecting the presence of a hexose in complex compounds, such as nucleic acid. Levulinic acid forms large glistening crystals, easily soluble in water. It forms a very characteristic silver salt.

UNSATURATED ALCOHOLS, ALDEHYDES, AND ACIDS.

ALLYL alcohol, acrolein, and acrylic acid are unsaturated compounds and the first members of the series :—

$$
\begin{array}{ccc}
\text{CH}_2\text{OH} & \text{CHO} & \text{COOH} \\
| & | & | \\
\text{CH} & \text{CH} & \text{CH} \\
\| & \| & \| \\
\text{CH}_2 & \text{CH}_2 & \text{CH}_2 \\
\text{Allyl alcohol.} & \text{Acrolein.} & \text{Acrylic acid.}
\end{array}
$$
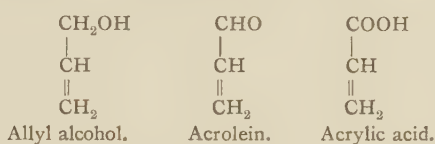
Amongst the unsaturated acids there are several which occur in nature.

Allyl Alcohol.

Preparation.

Allyl alcohol is prepared by distilling glycerol at a temperature of about 260° with oxalic acid. As stated on p. 93, the acid oxalic ester of glycerol is formed ; on heating it to a high temperature, the neutral ester is produced and decomposed, yielding allyl alcohol and carbon dioxide :—

$$
\begin{array}{l}
\text{CH}_2\text{OH} \qquad\quad \text{CH}_2\text{O}-\text{CO} \\
| \qquad\quad \text{HOOC} \quad | \qquad | \\
\text{CHOH} + \qquad | = \text{CHOH} \;\; \text{COOH} + \text{H}_2\text{O} \\
| \qquad\quad \text{HOOC} \quad | \\
\text{CH}_2\text{OH} \qquad\quad \text{COOH.}
\end{array}
$$

$$
\begin{array}{l}
\text{CH}_2\text{O}-\text{CO} \qquad \text{CH}_2\text{O}-\text{CO} \\
| \qquad | \qquad\quad | \qquad | \\
\text{CHOH} \;\; \text{COOH} = \text{CHO}-\text{CO} + \text{H}_2\text{O} \\
| \qquad\qquad\qquad | \\
\text{CH}_2\text{OH} \qquad\qquad \text{CH}_2\text{OH.}
\end{array}
$$

$$
\begin{array}{l}
\text{CH}_2\text{O}-\text{CO} \quad \text{CH}_2 \\
| \qquad | \qquad \| \\
\text{CHO}-\text{CO} = \text{CH} + 2\text{CO}_2 \\
| \qquad\qquad | \\
\text{CH}_2\text{OH} \qquad \text{CH}_2\text{OH.}
\end{array}
$$
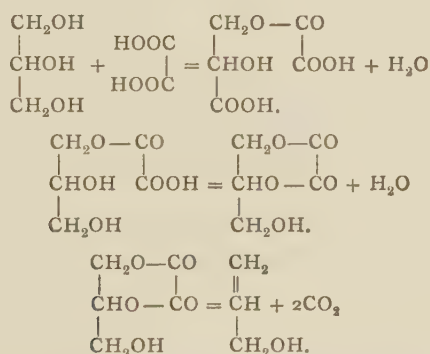
The liquid which distils over between 220 and 260° is collected and redistilled, the thermometer being placed in the liquid. Allyl alcohol is present in the fraction boiling below 105°. It is dehydrated with potassium carbonate and again distilled.

Properties.

Allyl alcohol is ·a colourless neutral liquid with an irritating smell and boils at 96-97°.   It mixes with water, alcohol, and ether in all proportions.

It has the properties of a primary alcohol and of an unsaturated compound, e.g. it reacts with sodium, forms esters, and yields acrolein and acrylic acid on oxidation; it combines with 2 atoms of chlorine, or bromine.

Esters of Allyl Alcohol.

Allyl iodide.—This ester may be prepared from allyl alcohol, but is more conveniently prepared from glycerol by the action of phosphorus and iodine.   The glycerol is probably converted into the tri-iodide, which decomposes into iodine and allyl iodide.

It is a colourless liquid boiling at 101° with the odour of garlic.

Allyl sulphide.—Allyl sulphide occurs in garlic and other plants, and is obtained by distilling the plant after it has been macerated with water.

It may be prepared from allyl iodide by heating with potassium sulphide in alcoholic solution (cf. p. 76).

It is a colourless, oily liquid boiling at 140° with the smell of garlic and hence is termed oil of garlic.

Allyl isothiocyanate is a constituent of black mustard seeds and is termed oil of mustard.

Acrolein.

Preparation.

Acrolein is prepared by distilling glycerol (1 part) with potassium hydrogen sulphate (2 parts) :—

$$\begin{array}{ccc} CH_2OH & CHO & \\ | & | & \\ CHOH & = CH & + 2H_2O \\ | & \| & \\ CH_2OH & CH_2 & \end{array}$$
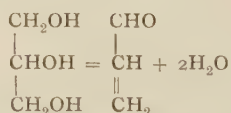
* The smell of acrolein is noticed on heating a few drops of glycerol in a dry test tube with anhydrous phosphoric acid, or acid potassium sulphate.

Properties.

Acrolein is a colourless liquid boiling at 52°, with a most irritating and peculiar odour; it affects the eyes, producing tears, and it forms sores upon the skin.

It has the reactions of an aldehyde, and also the reactions of unsaturated compounds.

Unsaturated Fatty Acids.

These acids contain in their molecule one or more pairs of their carbon atoms linked together by a double bond.   Unsaturated acids containing a triple bond are also known.

Acrylic acid is the simplest and first member of the homologous series of unsaturated acids containing one double bond.   It was first obtained by the oxidation of acrolein with silver oxide, but is more easily prepared by the following reactions :—

$$\overset{\displaystyle CH_2}{\underset{\displaystyle CHO}{\overset{\displaystyle \|}{\underset{\displaystyle |}{CH}}}} \xrightarrow{HCl} \overset{\displaystyle CH_2Cl}{\underset{\displaystyle CHO}{\overset{\displaystyle |}{\underset{\displaystyle |}{CH_2}}}} \xrightarrow[O]{HNO_3} \overset{\displaystyle CH_2Cl}{\underset{\displaystyle COOH}{\overset{\displaystyle |}{\underset{\displaystyle |}{CH_2}}}} \longrightarrow \overset{\displaystyle CH_2}{\underset{\displaystyle COOH}{\overset{\displaystyle \|}{\underset{\displaystyle |}{CH}}}}$$
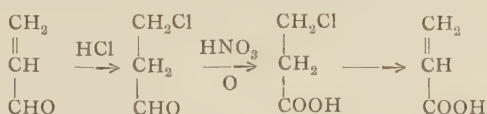
Acrylic acid is a liquid with a pungent smell and boils at 140°.

The next member is *crotonic acid*, $CH_3 . CH=CH . COOH$.

Crotonic acid is a solid which melts at 72°.

Oleic acid, which contains 18 carbon atoms and the double bond in the middle of the chain, is present in combination with glycerol in animal and vegetable fats from which it is prepared.

Oleic acid at the ordinary temperature is a colourless, oily liquid of sp. gr. ·900 at 11·8° with neither smell nor taste.   It oxidises very readily in the air, becoming brown, acid in reaction, and rancid in smell.   It can be frozen to a white crystalline solid which melts at 14°. It cannot be distilled at the ordinary temperature, but at 10 mm. pressure it distils at 223° and it is volatile with superheated steam.

Linoleic acid also contains 18 carbon atoms, but two double bonds. It is contained in linseed and other oils.

Linoleic acid resembles oleic acid, but is more readily oxidised by the oxygen of the air.   It is owing to its presence and that of other more unsaturated acids in linseed, cotton seed, and rape seed oils that these oils possess the property of forming the so-called " drying oils ". Oxygen is absorbed and transparent resinous substances are formed.

The salts of the unsaturated fatty acids are more soluble than those of the saturated fatty acids.   The lead and mercury salts of oleic acid are soluble in ether and are used for separating the mixture of acids obtained from fats.

Owing to the presence of the double bonds, the unsaturated acids combine by addition with the halogens, halogen acids, etc., and reduce permanganate solution becoming oxidised ; thus—

*   If a solution of oleic acid in chloroform be treated with bromine dissolved in chloroform, or iodine dissolved in chloroform containing

also mercuric chloride, the colour of the halogen is discharged until the acid is completely saturated by absorption of the halogen.

If a solution of sodium oleate be poured into a solution of potassium permanganate, the colour of the permanganate disappears and manganese dioxide separates out.

Fumaric and Maleic Acids.

Two isomeric unsaturated dicarboxylic acids of the formula,

$$HOOC . CH{=}CH . COOH,$$

are known.   They are fumaric and maleic acids.

Fumaric acid is contained in many fungi.   Its name has been derived from its presence in the juice of *Fumaria officinalis*.

Maleic acid has not been found in nature.

Both acids are formed on heating malic acid :—

$$HOOC . CHOH . CH_2 . COOH = H_2O + HOOC . CH{=}CH . COOH.$$
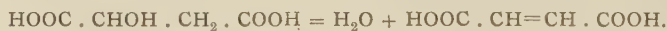
At low temperatures the chief product is fumaric acid.   If the product of the reaction be distilled, maleic acid passes over into the distillate as maleic anhydride.

The above constitution for these acids is shown by their chemical behaviour :—

(1) on reduction, they yield succinic acid,

(2) with hydrobromic acid, they yield monobromosuccinic acid,

(3) on heating with water, they yield inactive malic acid,

(4) on oxidation with permanganate, they yield tartaric acid ; fumaric acid gives the racemic acid, maleic acid gives mesotartaric acid.

Fumaric acid crystallises in needles, is soluble with difficulty in water, and sublimes without melting at 200°.   At high temperatures it changes into maleic anhydride.

Maleic acid crystallises in prisms, is easily soluble in water, and melts at 130°.   Other differences in their physical properties and in various derivatives have been observed.   It is easily changed into an anhydride.

It is of interest to notice also that fumaric acid is assimilated by moulds and bacteria, whilst maleic acid is not attacked.

The differences between these two acids are not easily accounted for on stereo-chemical considerations.   In compounds containing double bonds the two atoms of carbon so united are joined by the sides of the tetrahedra.

One form will then have the COOH groups far apart (trans form),
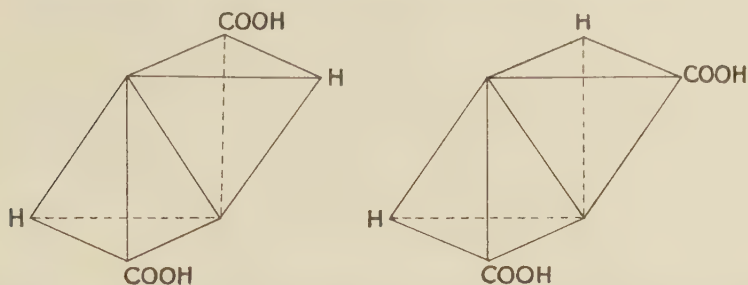
and the other form close together (cis form).   Projected on to a plane surface, the formulæ will be

$$HOOC \cdot C \cdot H$$
$$\|$$
$$H \cdot C \cdot COOH$$
Trans.
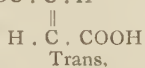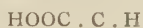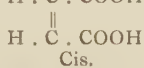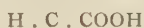
$$H \cdot C \cdot COOH$$
$$\|$$
$$H \cdot C \cdot COOH$$
Cis.

Maleic acid is the cis form, which easily forms an anhydride, and gives mesotartaric acid.

Fumaric acid is the trans form, does not form an anhydride and gives racemic acid.

CHAPTER XX.

THE amino acids are derivatives of the fatty acids, or of the dibasic acids, in which one or more of the hydrogen atoms in the chain are replaced by the NH_2 group. They are thus both amines and acids in their chemical nature. The amino acids are an exceedingly important group of compounds, since they are constituents of the proteins, in which they are combined together in various proportions (see p. 367). Amino acids have also been isolated from extracts of animal and vegetable tissues.

The amino acids are most conveniently arranged in eight groups according to the number of amino groups, or carboxyl groups, in the molecule, and further according to the presence, in addition, of hydroxyl (OH) groups, or thio (SH) groups, or aromatic and other radicles. The main characteristic is the presence of an amino group and a carboxyl group.

I. Monoaminomonocarboxylic Acids.

Glycine, or glycocoll, or amino-acetic acid :—

$$CH_2(NH_2) . COOH.$$

Alanine, or a-aminopropionic acid :—

$$CH_3 . CH(NH_2) . COOH.$$
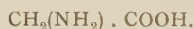
Valine, or a-aminoisovalerianic acid :—

$$\begin{matrix} CH_3 \\ \diagdown \\ \diagup \\ CH_3 \end{matrix} CH . CH(NH_2) . COOH.$$

Leucine, or a-aminoisocaproic acid :—

$$\begin{matrix} CH_3 \\ \diagdown \\ \diagup \\ CH_3 \end{matrix} CH . CH_2 . CH(NH_2) . COOH.$$

Isoleucine, or β-methyl-β-ethyl-a-aminopropionic acid :—

$$\begin{matrix} CH_3 \\ \diagdown \\ \diagup \\ C_2H_5 \end{matrix} CH . CH(NH_2) . COOH.$$
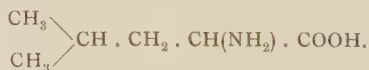
II. Monoaminodicarboxylic Acids.

Aspartic acid, or aminosuccinic acid :—

$$HOOC . CH_2 . CH(NH_2) . COOH.$$

166

Glutamic acid, or a-aminoglutaric acid :—

$$HOOC . CH_2 . CH_2 . CH(NH_2) . COOH.$$

III. Diaminomonocarboxylic Acids.

Ornithine, or a-, δ-diaminovalerianic acid, formed by the decomposition of arginine (p. 301) :—

$$CH_2(NH_2) . CH_2 . CH_2 . CH(NH_2) . COOH.$$

Lysine, or a-, ϵ-diaminocaproic acid :—

$$CH_2(NH_2) . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH.$$

IV. Hydroxyamino Acids.

Serine, or β-hydroxy-a-aminopropionic acid :—

$$CH_2OH . CH(NH_2) . COOH.$$

Hydroxyglutamic acid :—

$$HOOC . CHOH . CH_2 . CH(NH_2) . COOH.$$
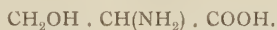
V. Thioamino Acids.

Cysteine, or β-thio-a-aminopropionic acid, formed by the decomposition of cystine, which is actually present in the protein molecule :—

$$CH_2SH . CH(NH_2) . COOH.$$

Cystine, or dicysteine, or di-(β-thio-a-aminopropionic acid) :—

$$HOOC . CH(NH_2) . CH_2 . S—S . CH_2 . CH(NH_2) . COOH.$$
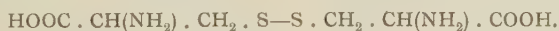
VI. Amino Acids with Aromatic Nucleus.

Phenylalanine, or β-phenyl-a-aminopropionic acid :—

$$C_6H_5 . CH_2 . CH(NH_2) . COOH.$$

Tyrosine, or β-parahydroxyphenyl-a-aminopropionic acid :—

$$HO . C_6H_4 . CH_2 . CH(NH_2) . COOH.$$
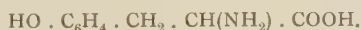
VII. Amino Acid with Indole Nucleus.

Tryptophan, or β-indole-a-aminopropionic acid (see p. 354) :—

$$C_8H_6N . CH_2 . CH(NH_2) . COOH.$$

VIII. Amino Acids with Heterocyclic Nuclei.

Histidine, or β-iminazole-a-aminopropionic acid :—

$$
\begin{array}{c}
CH \\
HN \diagdown N \\
HC = C—CH_2 . CH(NH_2) . COOH.
\end{array}
$$

Proline, or a-pyrrolidine carboxylic acid :—

$$
\begin{array}{c}
CH_2—CH_2 \\
CH_2 \quad CH . COOH \\
NH.
\end{array}
$$
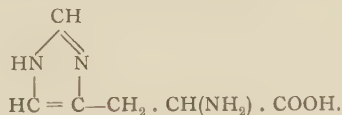
Hydroxyproline, or γ-hydroxy-α-pyrrolidine carboxylic acid :—

$$HO . CH—CH_2$$
$$CH_2 \quad CH . COOH$$
$$NH.$$
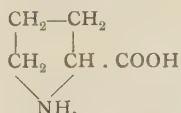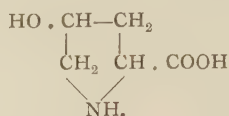
It should be noted that all these amino acids are α-amino derivatives of acids, i.e. derivatives in which a hydrogen atom in the α-carbon atom next to the COOH group has been substituted.

Preparation.

The amino acids are prepared by two general methods :—

(1) By the action of ammonia upon the corresponding halogen derivative :—

$$CH_3 \qquad\qquad CH_3$$
$$CHBr + NH_3 = HCl + CH . NH_2$$
$$COOH \qquad\qquad COOH$$
$$\text{Alanine.}$$
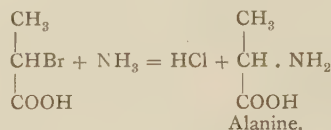
(2) By the addition of hydrogen cyanide and ammonia to aldehydes and the subsequent hydrolysis of the aminocyanohydrin :—

$$CH_3 \qquad\qquad CH_3$$
$$\qquad + NH_3 = \qquad /NH_2$$
$$CHO \qquad\qquad HC$$
$$\qquad\qquad\qquad \backslash OH$$

$$CH_3 \qquad\qquad\qquad CH_3$$
$$HC\,<^{NH_2}_{OH} + HCN = HC\,<^{NH_2}_{CN} + H_2O$$

$$CH_3 \qquad\qquad\qquad CH_3$$
$$HC—NH_2 + 2H_2O = HC—NH_2 + NH_3$$
$$CN \qquad\qquad\qquad COOH$$
$$\text{Alanine.}$$
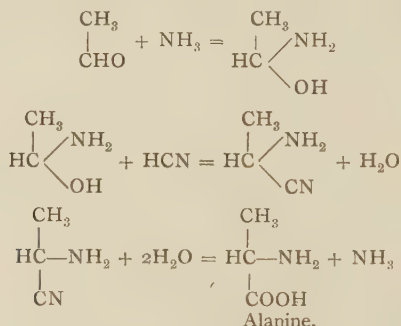
These reactions cannot always be used in the synthesis of amino acids since the halogen derivative, or aldehyde, is sometimes difficult to obtain.   Indirect methods have therefore been used.   The amino acid is not always prepared most easily by synthesis, but by the hydrolysis of proteins, e.g. tyrosine, cystine, etc.

Properties.

The amino acids in a pure state are white crystalline substances having characteristic forms.   Glycine and leucine crystals are shown in Figs. 24 and 25.

They are usually easily soluble in water (cystine and tyrosine are exceptions) but insoluble in alcohol (proline and hydroxyproline are

exceptions) and ether. They have generally high melting-points and decompose when they melt, losing carbon dioxide.

The monoaminomonocarboxylic acids containing one amino group and one acid group are neutral, the basic group neutralising the acid group. The diamino acids with two amino groups are basic. The dicarboxylic acids with two acid groups and one basic group are acidic in character.

The monoaminomonocarboxylic acids, which have a neutral reaction, possess a sweet taste, which is different for the different substances, and serves (with experienced workers) as a useful guide for defining the particular amino acid present in a solution.

FIG. 24.—Glycine.   FIG. 25.—Leucine.
(From Funke's " Atlas of Physiological Chemistry.")
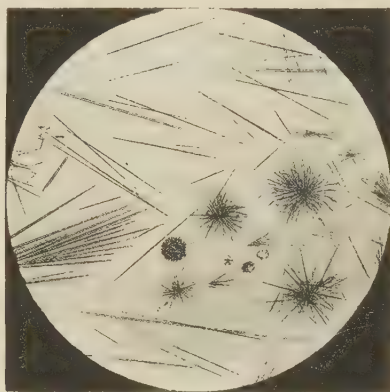
Except glycine, the amino acids are optically active; some are dextrorotatory, others laevorotatory. An inactive mixture is formed by synthesis. In all cases it has been separated into its stereoisomers.

The reactions of glycine are typical of the reactions of all the amino acids except cystine (p. 174), tyrosine (p. 211), tryptophan (p. 354) which contain other characteristic groups.

Glycine.

Preparation.

Glycine may be prepared by either of the general methods for synthesising amino acids :—

$$\begin{array}{c} CH_2Cl \\ | \\ COOH \end{array} + NH_3 = \begin{array}{c} CH_2.NH_2 \\ | \\ COOH \end{array} + HCl$$

$$\begin{array}{c} H \\ | \\ CHO \end{array} \xrightarrow{HCN} \begin{array}{c} H \\ | \\ CH \end{array}\begin{array}{c} OH \\ \\ CN \end{array} \xrightarrow{NH_3} \begin{array}{c} H \\ | \\ CH \end{array}\begin{array}{c} NH_2 \\ \\ CN \end{array} \xrightarrow{Ba(OH)_2} \begin{array}{c} H \\ | \\ CH \end{array}\begin{array}{c} NH_2 \\ \\ COOH. \end{array}$$
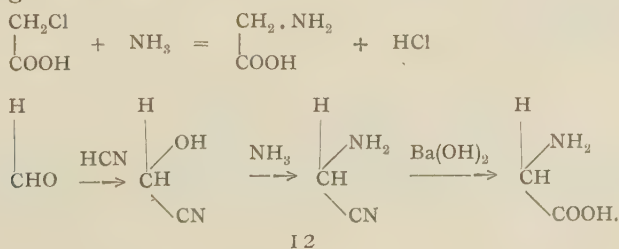
It is fairly easily obtained by the hydrolysis of the protein, gelatin, with conc. HCl as follows :—

100-200 grams of gelatin are dissolved by warming in 300-600 c.c. of conc. hydrochloric acid and then boiled under a reflux condenser for 6 hours.   The dark brown solution is evaporated *in vacuo* to a syrup. The syrup is dissolved in 500-600 c.c. absolute alcohol and saturated with dry hydrochloric acid gas.   The alcohol is removed by distillation *in vacuo*, and esterification is repeated by adding 500 c.c. absolute alcohol and again saturating with dry gaseous hydrochloric acid.   On cooling and standing, glycine ester hydrochloride crystallises out.   It is filtered off and washed with a little alcohol.   It is converted into the ester by dissolving in a small quantity of water, saturating the water with anhydrous solid sodium carbonate and extracting with ether.   The ethereal solution is dried with sodium sulphate and the ether removed by distillation.   The ester is then distilled *in vacuo*.   On boiling the ester with water under a reflux condenser until the reaction is neutral and evaporating the solution to a small bulk, glycine crystallises out.

Properties.

A. *As Acid.*

(1) Glycine forms salts with bases :—

$$CH_2 . NH_2 \qquad NH_2 \qquad NH_2$$
$$\mid \qquad\qquad \mid \qquad\qquad \mid$$
$$COONa \qquad CH_2 . COO—Cu—OOC . CH_2.$$
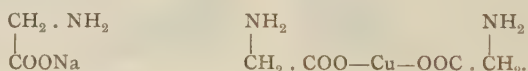
The copper salt is the most characteristic salt of amino acids ; it has a somewhat deep blue colour and generally crystallises well, so that it serves for the isolation of amino acids and for their identification by a determination of the copper content.

*   On boiling a solution of glycine with excess of copper hydrate, or copper carbonate, filtering off the excess and evaporating the solution until it crystallises and then allowing to cool, the blue copper salt of glycine crystallises out.

*   The depth of colour of the copper salt of glycine can be seen by adding a few drops of copper sulphate solution to a solution of glycine. The shade of colour is different to that of copper sulphate and in comparison deeper.

(2) Glycine forms esters with alcohols :—

$$CH_2 . NH_2 \qquad\qquad CH_2 . NH_2$$
$$\mid \qquad\qquad\qquad\qquad \mid \qquad + H_2O.$$
$$COOH + C_2H_5OH = \qquad COOC_2H_5$$
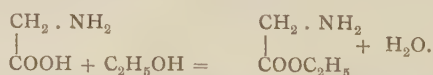
These esters are prepared by passing dry hydrogen chloride gas into a suspension of the amino acid in absolute alcohol.   The amino acid is

converted into its hydrochloride, dissolves, and is esterified, so that the hydrochloride of its ester is obtained :—

$$CH_2 . NH_2 . HCl$$
$$|$$
$$COOC_2H_5.$$
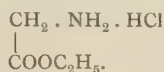
The ester is obtained by evaporating off the alcohol, making alkaline with sodium carbonate, extracting the ester with ether, drying with sodium sulphate, distilling off the ether, and finally distilling the ester *in vacuo* :—

$$2 \begin{array}{c} CH_2 . NH_2 . HCl \\ | \\ COOC_2H_5 \end{array} + Na_2CO_3 = 2 \begin{array}{c} CH_2 . NH_2 \\ | \\ COOC_2H_5 \end{array} + NaCl + CO_2 + H_2O.$$

The esters of amino acids are generally oily liquids having an alkaline reaction. They can be distilled *in vacuo*. The complex mixture of amino acids which results on the hydrolysis of proteins is separated by fractional distillation of the esters *in vacuo*.

B. *As Amine.*

(1) Glycine forms salts with acids, e.g. :—

$$CH_2 . NH_2 . HCl$$
$$|$$
$$COOH.$$
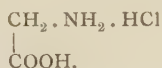
These salts are generally crystalline and very easily soluble in water and are acid in reaction.

*   (2) Ammonia is not evolved on boiling an amino acid with sodium hydroxide (compare acid amides).

On boiling a solution of glycine with caustic soda, no evolution of ammonia can be detected by smell, litmus paper, etc.

*   (3) Like amines, amino acids are decomposed by nitrous acid. On adding dilute acetic acid and a few drops of sodium nitrite solution to a solution of glycine, there is an evolution of nitrogen :—

$$\begin{array}{c} CH_2 . NH_2 \\ | \\ COOH \end{array} + HNO_2 = \begin{array}{c} CH_2 . OH \\ | \\ COOH \end{array} + N_2 + H_2O.$$
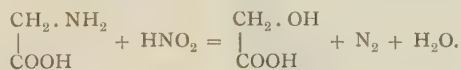
The corresponding hydroxy acid is formed.

(4) On treating with acid chlorides, the amino acids yield substituted amides :—

$$\begin{array}{c} CH_2 . NH_2 \\ | \\ COOH \end{array} + CH_3COCl = \begin{array}{c} CH_2 . NH . OC . CH_3 \\ | \\ COOH \end{array}$$

Hippuric Acid, $C_6H_5CO—NH . CH_2 . COOH.$

Hippuric acid, or benzoyl glycine, is formed synthetically by the kidney when benzoic acid is injected into the blood-stream of animals.

12 *

It is a compound which is normally present in the urine of animals, especially herbivora. · Benzoic acid is formed from the aromatic substances of the food and is converted into hippuric acid during its excretion.

Preparation.

(1) The benzoyl derivatives of amino acids are readily formed by shaking the amino acid in solution in sodium bicarbonate with benzoyl chloride :—

$$\begin{array}{ccc} \begin{array}{l} CH_2 . NH_2 \\ | \\ COOH \end{array} & + C_6H_5COCl = & \begin{array}{l} CH_2 . NH . OC . C_6H_5 \\ | \\ COOH \end{array} \\ & \begin{array}{c} \text{Benzoyl} \\ \text{Chloride.} \end{array} & \begin{array}{c} \text{Benzoyl glycine} \\ \text{or} \\ \text{hippuric acid.} \end{array} \end{array}$$
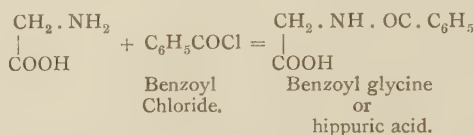
(2) Hippuric acid is formed by the action of chloracetic acid upon benzamide :—

$$\begin{array}{l} CH_2Cl \\ | \\ COOH \end{array} + H_2N . CO . C_6H_5 = \begin{array}{l} CH_2 . HN . CO . C_6H_5 \\ | \\ COOH \end{array} + HCl$$
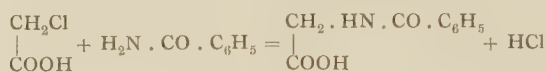
(3) *From Urine.*

Hippuric acid is readily obtained from herbivorous urine by adding 1 c.c. of concentrated hydrochloric acid and 12 gm. of ammonium sulphate to every 25 c.c. The compound commences to crystallise out in about 5 minutes and the crystallisation is complete in 10-15 minutes. The crystals are filtered off and dried. They are generally more or less pigmented and are purified by recrystallisation from boiling water with the addition of a small quantity of animal charcoal. On filtering and cooling the filtrate, hippuric acid crystallises out in the form of needles (Fig. 26).

Fig. 26.—Hippuric acid.   (After Funke.)

Properties and Reactions.

Hippuric acid is a white crystalline substance which melts at 187·5°. It is soluble with difficulty in cold water, but readily in hot water, in alcohol, and ethyl acetate. It is only slightly soluble in ether and chloroform and insoluble in petroleum ether (this distinguishes it from benzoic acid).

*   (1) On heating, hippuric acid melts; the mass on further heating turns reddish-brown, due to the decomposition of the glycine, and a smell of bitter almonds is produced. A sublimate of benzoic acid is also formed. If this be dissolved in dilute sodium carbonate solution and the solution be acidified with dilute hydrochloric acid, benzoic acid is precipitated. The crystals have a different appearance to hippuric acid under the microscope and give the reactions for benzoic acid (p. 198).

*   (2) Ammonia is given off on heating hippuric acid with soda lime.

*   (3) On adding ferric chloride to a neutral solution of hippuric acid, a reddish-brown precipitate is formed; this is soluble in hydrochloric acid and the solution will deposit crystals of hippuric acid.

*   (4) Hippuric acid is hydrolysed into benzoic acid and glycine by boiling with concentrated hydrochloric acid :—

$$C_6H_5CO—NH . CH_2 . COOH + H_2O = C_6H_5COOH + H_2N . CH_2 . COOH.$$

The benzoic acid crystallises out on cooling the solution and is separated by filtration.

The presence of glycine in the solution may be shown by adding a slight excess of ammonia, boiling the solution till neutral, and adding a few drops of copper sulphate solution; the deep blue colour characteristic of the copper salt of glycine is formed.

Glutamic Acid.

This amino acid is contained in large amounts in the proteins of cereals from which it is easily prepared.

Wheat gluten is made by mixing flour with water to form a dough, placing the dough in muslin, and kneading it carefully in running water till the starch has been removed. A sticky yellowish mass of gluten remains. It is dried on the water-bath.

100 gm. of gluten are boiled under a reflux for 6 hours with conc. HCl. The solution is filtered and evaporated to a small volume. On saturating the solution with dry hydrochloric acid gas and allowing to stand, glutamic acid hydrochloride crystallises out. It is filtered off, and washed with conc. HCl and dried. Glutamic acid is made by adding the calculated quantity of soda and fractionally crystallising.

$$HOOC . CH_2 . CH_2 . CH(NH_2) . COOH + NaOH = HOOC . CH_2 . CH_2 . CH(NH_2)COOH$$
$$\underset{HCl}{|} \qquad\qquad\qquad + NaCl + H_2O$$
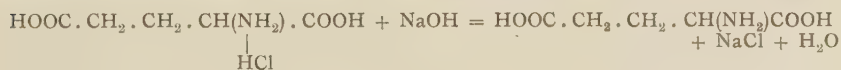
Cystine.

The amino acid, cystine, is present in greatest amount in the proteins, keratins. It has been found in the liver and other organs and

occasionally forms concretions in the bladder and deposits in the urine (cystinuria). The amount excreted in these conditions is small, but from 0·5-1 gm. have been recorded *per diem*.

Preparation.

* Cystine is prepared by the hydrolysis of keratins such as wool, hair feathers, most easily by Folin's method. The wool, or hair, is hydrolysed by boiling under a reflux condenser with concentrated hydrochloric acid for about five hours in the proportion of 50 gm. wool to 100 c.c. acid.

The dark brown solution is filtered from black humin matter and evaporated *in vacuo* to remove HCl as far as possible. The residue is dissolved in water and partially neutralised with soda. Solid sodium acetate is then slowly added to the hot solution until the reaction of the solution is no longer acid to congo red. A dark precipitate containing the cystine comes down on cooling. The precipitate is filtered off when the solution is cold and washed with cold water. It is dissolved in 5 per cent. hydrochloric acid, filtered from tarry matter, and the solution is boiled with animal charcoal till it is colourless. The solution is filtered whilst hot and hot sodium acetate solution added until it is neutral to congo red. Cystine crystallises out in the typical hexagonal plates on cooling.

Properties.

Cystine crystallises in colourless hexagonal plates, or prisms (Fig. 27).
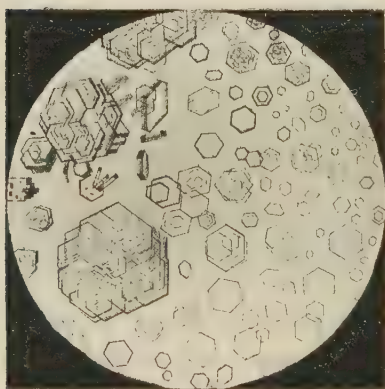
FIG. 27.—Cystine. (After Funke.)

It is almost insoluble in water, alcohol, and ether. It dissolves in dilute acids and in ammonia and in solutions of caustic alkali and alkali carbonates. It crystallises from its solution in ammonia in the typical hexagonal plates when the solution is allowed to evaporate. Crystals for microscopic examination and identification by crystalline form are conveniently obtained in this way on a microscopic slide.

If cystine be heated on platinum foil it burns with a bluish-green flame without melting.

The sulphur in the molecule of cystine is held in loose combination and is partially evolved as hydrogen sulphide on boiling with alkali. It is thus readily distinguished from other amino acids.

* On dissolving some cystine in caustic alkali, or on adding caustic alkali to a solution of cystine, to which a drop of lead acetate solution has been added and boiling, a brownish, or black, precipitate of lead sulphide is formed.

Taurine.

Taurine, or aminoethyl sulphonic acid, is an amino acid containing sulphuric acid instead of carboxyl as the acid group. It has not been found as a constituent of proteins, but is probably derived from cystine, or cysteine, which is converted by oxidation in the animal body into taurine :—

$$
\begin{array}{lll}
CH_2 . SH & CH_2 . SO_3H & CH_2 . SO_3H \\
| & | & | \\
CH . NH_2 \rightarrow & CH . NH_2 & \rightarrow CH_2 . NH_2 + CO_2 \\
| & | & \\
COOH & COOH & \\
\text{Cysteine.} & \text{Cysteic acid.} & \text{Taurine.}
\end{array}
$$
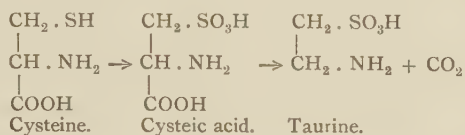
Taurine has been isolated from the lungs and kidneys of oxen and from the muscles of invertebrates. In combination with cholalic acid as taurocholic acid, it is present in bile.

Preparation.

Taurine is most easily prepared from ox bile by boiling it for some hours with dilute hydrochloric acid. The filtrate from the insoluble anhydrides of the bile acids is concentrated on the water-bath to a small volume and filtered whilst hot from sodium chloride, etc. The solution is evaporated to dryness and the residue dissolved in 5 per cent. hydrochloric acid. It is precipitated from solution by adding 10 volumes of 95 per cent. alcohol. The crystals are purified by solution in acid, precipitation by alcohol and recrystallisation from water.

FIG. 28.—Taurine. (After Funke.)

Properties.

Taurine crystallises in colourless four- or six-sided prisms (Fig. 28). It is soluble in 15-16 parts of cold water, more easily in hot water. It is insoluble in absolute alcohol and ether, but is slightly soluble in cold dilute alcohol, more easily in hot.

It is decomposed on boiling with caustic alkali yielding acetic and sulphurous acids.

It is identified by its crystalline form, sulphur content, and the formation of a white insoluble compound when its solution is boiled with freshly precipitated mercuric oxide. It is not precipitated by metallic salts.

Estimation of Amino Acids.

(1) *By Titration.*

As the amino acids are neutral in reaction, they cannot be titrated by means of standard alkali like ordinary acids. They combine, however, with formaldehyde and yield an acid which can be titrated in this way :—

$$
\begin{array}{ccc}
\underset{\substack{|\\ \text{COOH}}}{\text{CH}_2 \cdot \text{NH}_2} & + \text{ OHC} \cdot \text{H} = & \underset{\substack{|\\ \text{COOH}}}{\text{CH}_2 \cdot \text{N}{=}\text{CH}_2} + \text{ H}_2\text{O}.
\end{array}
$$
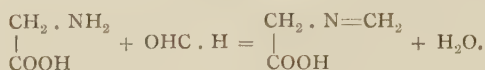
The process of estimation is carried out as follows :—

* 10 c.c. of commercial formalin are diluted with 2 volumes of water and neutralised with $\cdot 1 \text{N}$ alkali, using 6-8 drops of phenolphthalein in the solution as indicator. This neutralised formalin is added to 20 or 25 c.c. of the amino acid solution measured out with a pipette into a small flask, or beaker. The pink colour disappears. $\cdot 1 \text{N}$ sodium hydroxide solution is run in from a burette until a distinct pink colour again appears. The number of c.c. of alkali used is noted.

If the amino acid be known, its amount in the solution can be calculated :—

$$
\underbrace{\text{CH}_2 \cdot (\text{NH}_2) \cdot \text{COOH}}_{75} + \underbrace{\text{NaOH}}_{40} = \text{CH}_2 \cdot (\text{NH}_2) \cdot \text{COONa} + \text{H}_2\text{O}.
$$

1 c.c. $\cdot 1 \text{N}$ NaOH = 1 c.c. $\cdot 1 \text{N}$ glycine = 0·0075 gm. glycine.
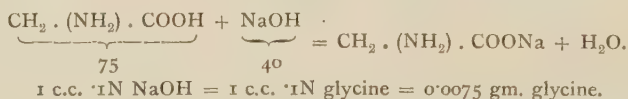
If the amino acid be unknown, or if a mixture of amino acids be present, the amount is best expressed in terms of $\cdot 1 \text{N}$ acid, or in terms of nitrogen.

(2) *By estimation of the amino nitrogen.*

Amino acids can be readily estimated by the measurement of the volume of nitrogen evolved by the action of nitrous acid according to the equation :—

$$
\begin{array}{ccc}
\underset{\substack{|\\ \text{COOH}}}{\text{CH}_2 \cdot \text{NH}_2} & + \text{ HNO}_2 = & \underset{\substack{|\\ \text{COOH}}}{\text{CH}_2\text{OH}} + \text{ N}_2 + \text{ H}_2\text{O}.
\end{array}
$$
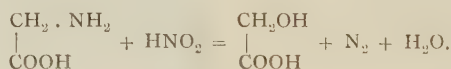
Half of the volume of nitrogen evolved corresponds to the amount of nitrogen in the amino acid.

A most convenient apparatus for this estimation has been devised by Van Slyke.

AROMATIC compounds are mostly contained in the fragrant and peculiar smelling oils, resins, etc., which are present in the flowers, leaves, and other parts of plants and which often ooze out when the bark is broken. Turpentine, india-rubber, tannin, oil of lemon, oil of cloves, cinnamon, and numerous plant pigments are aromatic compounds. They derive their name from their origin in these sweet-smelling natural substances.

Though only a few aromatic compounds are actually found in animals, yet they are essential to their life.

Another great source of aromatic compounds is coal tar, which is a complex mixture, but contains benzene, the parent substance from which all the other compounds can be derived.

In some respects the aromatic resemble the fatty, or aliphatic, compounds, but in other respects they are totally different. They differ by having a greater carbon content and in being more resistant to oxidation, reduction, etc. The more complex substances can be oxidised and converted into simpler substances, but these simpler substances are resistant and are found to possess a stable nucleus composed of six atoms of carbon. This nucleus is not easily oxidised to compounds containing fewer carbon atoms, but on oxidation it is converted into carbon dioxide and water. The presence of a nucleus of six carbon atoms is thus a characteristic of aromatic compounds.

Another characteristic is the formation of nitro compounds and sulphonic acid derivatives by the direct action of nitric and sulphuric acids.

The Structure of Aromatic Compounds.

Our representation of the structure of the aromatic compounds is based upon the theory of Kekulé, which was published in 1865.

Benzene, C_6H_6, is the simplest aromatic compound containing six carbon atoms. Its general formula is C_nH_{2n-6}, which indicates a higher degree of unsaturation than is shown by either the ethylene, or acetylene,

series of hydrocarbons.   Yet its properties do not correspond with the properties of the unsaturated hydrocarbons such as :—

$$CH\equiv C-CH_2-CH_2-C\equiv CH.$$
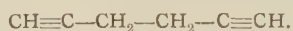
but point to the presence of six CH groups, arranged symmetrically in the molecule.   Kekulé suggested that the six carbon atoms were united in a *closed* chain, or ring, and joined by alternate single and double bonds, a hydrogen atom being united to each carbon atom.   The tetravalency of the carbon atom is thus satisfied and a symmetrical structure results, thus :—

$$
\begin{array}{c}
\text{H} \\
\text{C} \\
\text{HC} \quad \text{CH} \\
\text{HC} \quad \text{CH} \\
\text{C} \\
\text{H}
\end{array}
$$
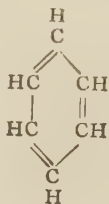
The symmetry of the molecule is better represented by a regular hexagon at each angle of which there is a CH group ; thus :—

$$
\begin{array}{c}
\text{CH} \\
\text{HC} \qquad \text{CH} \\
\text{HC} \qquad \text{CH} \\
\text{CH}
\end{array}
$$
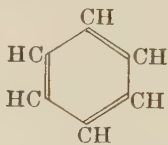
This structure shows that the six hydrogen atoms are of equal value.   Only one monosubstitution derivative is theoretically possible, and in accordance with experiment, only one monosubstitution derivative can be produced.   There are no isomeric monosubstituted derivatives of benzene.

It explains the existence of three isomeric disubstituted derivatives :—

$$
\begin{array}{ccc}
\text{X} & \text{X} & \text{X} \\
\hexagon\text{X} & \hexagon & \hexagon \\
 & \text{X} & \text{X}
\end{array}
$$

Ortho-   Meta-   Para-
o-.   *m*-.   *p*-.

and of three isomeric trisubstituted derivatives :—

$$
\begin{array}{ccc}
\text{X} & \text{X} & \text{X} \\
\hexagon\text{X} & \hexagon\text{X} & \hexagon \\
\text{X} & \text{X} & \text{X X}
\end{array}
$$

The chief criticism against this formula is the alternate linking of the carbon atoms by double and single bonds. Their existence would point to the possibility of the existence of two isomeric monosubstitution derivatives and of more disubstitution derivatives. To overcome this difficulty Kekulé assumed that there was a continual alternation between the double and single bonds, and that the double bonds are not like the double bonds in unsaturated aliphatic compounds.

This formula further represents benzene as an unsaturated compound. In some respects benzene behaves like an olefine, but not in all respects, e.g. it forms addition compounds with the halogens and with hydrogen, but not with the halogen acids and sulphuric acid, nor does it decolorise permanganate in the cold.

Other structures have been proposed for the formula of benzene, such as a prism, but these do not explain the various substitution derivatives satisfactorily. The ring structure of a regular hexagon has been accepted by all chemists. In order to retain the tetravalency of the carbon atom, the centric formula was proposed by Armstrong.

The extra bonds making the carbon atoms tetravalent simply point to the centre of the figure, and come into play only under certain conditions, e.g. amongst the hydroaromatic compounds.
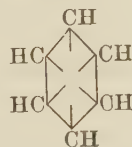
For simplification, the benzene nucleus is generally represented as a regular hexagon, at each angle of which the existence of a carbon atom and a hydrogen atom is recognised.

The substitution products are then indicated by introducing only the particular radicle, or radicles. Further, the carbon atoms are generally numbered in order. · The compound is 1-chloro-3-nitrobenzene.
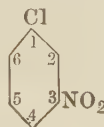
Coal Tar and its Constituents.

The dry distillation of coal gives primarily illuminating, or coal, gas and coke. The gas is passed through a series of vertical condensers and deposits, on cooling, a liquid. On standing, this liquid separates into two layers. The upper layer is ammoniacal gas liquor. The lower layer is coal tar. A ton of coal yields from 10-20 gallons of tar, or about 5 per cent. of its weight, but the yield varies according to the coal and to the temperature of the distillation. Higher temperatures give more gas, lower temperatures more coal tar.

Coal tar is a thick black liquid; its black colour comes chiefly from finely divided admixed particles of coal, which may make up 10-30 per cent. of the whole quantity. The remainder consists chiefly of aromatic compounds, which have arisen by condensation of carbon compounds of the fatty series by the high temperature of the distillation retorts. The constituents of coal tar are of three kinds :—

(1) The neutral—benzene, toluene, xylene, naphthalene, anthracene, etc.

(2) The acid—carbolic acid or phenol, cresols, and naphthols.

(3) The basic—aniline, pyridine, and quinoline.

They are separated from one another, after fractional distillation, by extraction with alkali and acid. The neutral remain behind.

Distillation of Coal Tar.

Coal tar is distilled in wrought-iron stills and the distillate is collected in the following fractions :—

(1) Light oil, or crude naphtha, which distils up to 170° and contains benzene, toluene, xylene. It forms 3-5 per cent.

(2) Middle oil, or carbolic oil, which distils from 170-230° and contains naphthalene and carbolic acid. It forms 8-10 per cent.

(3) Heavy oil, or creosote oil, which distils from 230-270° and forms 8-10 per cent.

(4) Anthracene oil, which distils from 270-400° and contains anthracene. It is coloured green and forms 16-20 per cent.

Pitch remains in the still.

The terms light oil, middle oil, heavy oil denote the specific gravity of the distillate with regard to water. A sample is run into water during the distillation; if it floats it is light oil, if it sinks it is heavy oil. The middle oil passes over as soon as light oil no longer distils over.

BENZENE AND ITS MONOSUBSTITUTION DERIVATIVES.

Benzene.

BENZENE was discovered by Faraday in 1825 in illuminating gas prepared from oil, before the introduction of coal gas. The gases were condensed to a liquid from which benzene was isolated by distillation.

Preparation.

Mitscherlich prepared benzene in 1834 by distilling benzoic acid with lime :—

$$\left. \begin{array}{c} C_6H_5COO \\ C_6H_5COO \end{array} \right\rangle Ca + Ca(OH)_2 = 2C_6H_6 + 2CaCO_3.$$
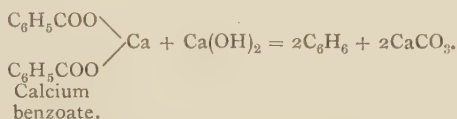
Calcium
benzoate.

A mixture of 5 gm. of calcium benzoate and twice its quantity of soda lime is heated in a test tube. The smell of benzene is noticed. On igniting, it will burn with a smoky flame.

With a larger quantity of calcium benzoate (30-50 gm.) the mixture is heated in a distilling flask connected with a condenser. The distillate is separated from water by means of a separating funnel, dried with calcium chloride, and distilled.

In 1845 it was found in coal tar by Hofmann. Berthelot obtained benzene by passing acetylene through a red-hot tube :—

$$3C_2H_2 = C_6H_6.$$

Benzene and its homologues toluene and xylenes are prepared from the light oil from coal tar. The fraction is shaken with strong sulphuric acid which removes basic substances, such as aniline, pyridine, and also unsaturated hydrocarbons. It is next shaken with sodium hydroxide which removes acid substances, carbolic acid, and the sulphuric acid. It is washed by shaking with water and fractionally distilled. Pure benzene is isolated from the first fraction by careful fractional distillation.

Properties.

Benzene is an oily colourless liquid with peculiar smell and boils at 80°. It solidifies on cooling and the crystals melt at 5·4°. Its

specific gravity is ·874 at 20°. It is inflammable and burns with a smoky and luminous flame.   It is insoluble in water, but mixes with alcohol and ether.   Benzene is a good solvent for resins, fats, etc., and is frequently used for extraction.   It dissolves iodine, phosphorus, sulphur, etc.

Benzene is called benzole commercially, the terminal *e* being added to show that it does not possess a hydroxyl (OH) group; the suffix *ol*, as previously mentioned, is used to designate alcohols.

<div align="center">

Chlorobenzene, C_6H_5Cl.

Bromobenzene, C_6H_5Br.

Iodobenzene, C_6H_5I.

</div>

Like the aliphatic hydrocarbons benzene is acted upon by the halogens, chlorine and bromine, and converted into substitution derivatives.   This reaction proceeds most easily in the presence of a halogen carrier, such as iodine (iodine chloride is formed which chlorinates more vigorously) :—

<div align="center">

$$\text{⬡} + Br_2 = HBr + \text{⬡}^{Br.}$$

</div>
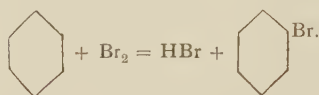
*   This reaction can be observed by adding a few drops of bromine to a few c.c. of benzene in a test tube.   The evolution of hydrobromic acid is slow.   If a piece of aluminium mercury couple, or some iron filings, be added to a few c.c. of benzene in another test tube and then a few drops of bromine, the reaction is more rapid.

Benzene is not acted upon by iodine, but iodobenzene has been prepared by heating benzene with iodine and iodic acid :—

<div align="center">

$5C_6H_6 + 4I + HIO_3 = 5C_6H_5I + 3H_2O.$

</div>
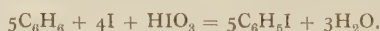
Iodobenzene is obtained through the diazo reaction (p. 191).

Properties.

These halogen derivatives are colourless liquids with a faint but not disagreeable smell.   They boil without decomposition, are insoluble in water but soluble in organic solvents.

Reactions.

These compounds are more stable than the corresponding aliphatic halogen compounds.   The halogen atom is *not* replaceable by OH groups, or other groups.

Nitrobenzene, $C_6H_5NO_2$.

Benzene is not acted upon by dilute nitric acid, but it is converted into nitrobenzene by the action of concentrated nitric acid (nitration) :—

$$\text{⬡} + HNO_3 = H_2O + \text{⬡}NO_2.$$
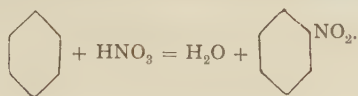
This reaction is one of the principal reactions which benzene and its derivatives undergo and in which aromatic compounds differ from aliphatic compounds.

Preparation.

* 3 or 4 drops of benzene are added to a mixture of 2 c.c. of concentrated sulphuric acid and 1 c.c. of concentrated nitric acid and warmed. Nitrobenzene is formed and is recognised by its smell of bitter almonds, which remains after removing the excess of acid by alkali.

On a larger scale nitrobenzene may be prepared as follows :—

100 gm. of concentrated nitric acid are added with shaking to 150 gm. of concentrated sulphuric acid in a 500 c.c. flask and cooled by placing under running water. 50 gm. of benzene are added in portions of 2 c.c. to the cold mixture of acids ; after each addition the mixture is thoroughly shaken. There is an energetic reaction and the contents of the flask must be kept below 50° by immersing it in cold water. The addition of benzene should take at least half an hour. The reaction is completed by heating the contents of the flask under an air condenser in a water-bath at 60°. The nitrobenzene floats as an oil on the surface. It is separated from the acid by means of a tap funnel and shaken with water. The heavier layer of nitrobenzene is separated, washed with excess of sodium carbonate solution to remove acid and again with water. It is dried by being shaken with calcium chloride, filtered through glass wool and distilled, using an air condenser, the fraction boiling from 204-208° being collected. A small residue of dinitrobenzene may remain in the flask.

Properties.

Nitrobenzene is a pale yellow liquid which possesses an odour of bitter almonds. It boils at 205° and can be frozen to a solid which melts at 3°. It is frequently used for scenting soap but chiefly in the preparation of aniline and benzidine : it is sometimes used as a solvent.

Benzene Sulphonic Acid, $C_6H_5 . SO_3H$.

Benzene slowly dissolves in warm concentrated sulphuric acid and is converted into benzene sulphonic acid (sulphonation) :—

$$\text{⬡} + \begin{matrix} HO \\ HO \end{matrix}\!\!>\!\!S\!\!<\!\!\begin{matrix} O \\ O \end{matrix} = H_2O + \text{⬡}S\!\!<\!\!\begin{matrix} O \\ =O. \\ OH \end{matrix}$$
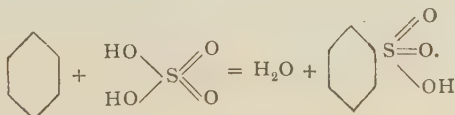
Preparation.

*   2 c.c. of benzene are mixed with 7 c.c. of concentrated sulphuric acid and carefully heated with constant shaking. The benzene which at first floats on the surface gradually dissolves. A clear solution is obtained on pouring a portion of the cooled mixture into water. Sodium benzene sulphonate separates, if some be poured into a saturated solution of sodium chloride.

On a larger scale the benzene and sulphuric acid are carefully heated under a reflux condenser with constant stirring. The reaction product is poured into water (or salt solution if the sodium salt be required) and the solution neutralised with calcium carbonate. The calcium sulphate is filtered off and the filtrate is evaporated until the calcium salt crystallises. The other salts, or the free acid, are prepared from the calcium salt by double decomposition with potassium carbonate, etc., or sulphuric acid.

Properties.

Benzene sulphonic acid is a hygroscopic solid which is readily soluble in water and melts at 50°. The solution is strongly acid.

It forms salts with the metallic carbonates, or oxides. These salts generally crystallise well.

On heating, the sulphonic acid is decomposed. The sulphonic acid group may be removed by heating it in a sealed tube with concentrated hydrochloric acid, or with strong sulphuric acid in a current of steam; the hydrocarbon is regenerated :—

$$C_6H_5SO_3H + H_2O = C_6H_6 + H_2SO_4.$$
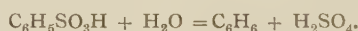
Note.—This reaction serves for the separation of a mixture of unsaturated hydrocarbon, an aromatic hydrocarbon, and a paraffin. The unsaturated hydrocarbon dissolves in cold sulphuric acid, the aromatic hydrocarbon forms the sulphonic acid with hot sulphuric acid, and the paraffin is not acted upon.

Benzene Sulphonyl Chloride.

Benzene sulphonic acid, or its salts, is converted by the action of phosphorus pentachloride into benzene sulphonyl chloride :—

$$C_6H_5 . SO_2OH + PCl_5 = HCl + POCl_3 + C_6H_5SO_2Cl.$$

The two substances are heated on a water-bath till hydrochloric acid is no longer evolved. The product is poured into water and the sulphonyl chloride extracted with ether. It is purified by distillation *in vacuo*.

Benzene sulphonyl chloride is a white solid which melts at 14° and boils at 116°. It has a pungent odour and is not rapidly decomposed by water.

By the action of ammonium carbonate upon benzene sulphonyl chloride *benzene sulphonamide* is formed :—

$$C_6H_5SO_2Cl + NH_3 = C_6H_5SO_2NH_2 + HCl.$$

Benzene sulphonyl chloride also reacts with aniline and primary amines, and secondary amines, but not with tertiary amines :—

$$C_6H_5SO_2Cl + H_2N.C_6H_5 = C_6H_5.SO_2.HN.C_6H_5 + HCl$$
<center>Benzene sulphonanilide.</center>
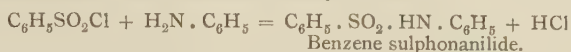
With alcohols, it forms esters :—

$$C_6H_5SO_2Cl + C_2H_5OH = C_6H_5SO_2.OC_2H_5 + HCl.$$
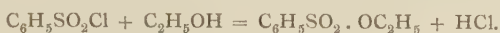
Phenol, or Carbolic Acid, C_6H_5OH.

Phenol is obtained by fusing benzene sulphonic acid with caustic potash :—
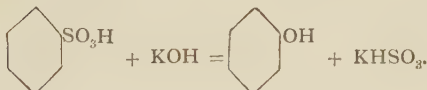
It is also obtained by the decomposition of diazobenzene (p. 191).

Preparation.

Phenol is contained in coal tar and is present in the middle oil fraction.   This fraction on cooling deposits crystals of naphthalene, which are filtered off and pressed out.   The oil is shaken with caustic soda which dissolves the phenol ; the alkaline layer is separated and treated with sulphuric acid.   Phenol separates out as an oil : it is washed with water and distilled.   The distillate separates into crystalline phenol and impure liquid phenol.   Pure crystals are obtained on re-distillation.

Properties.

Phenol crystallises in colourless prisms which are deliquescent and turn pink on contact with air and light.   It melts at 42° and boils at 182°.   It has a characteristic smell, is very poisonous, and has a marked caustic action upon the skin.   It is largely used as a disinfectant, either in 1-3 per cent. aqueous solution, or mixed with china clay or other absorbent powders.

Phenol is not easily soluble in water (1 part in 15 parts of water), but it dissolves in alcohol and other organic solvents.   It is volatile with steam.

Phenol is a tertiary alcohol ; on oxidation it is broken down and gives a variety of products.   It differs from aliphatic compounds containing hydroxyl groups in having acid properties.   Hence it is usually termed carbolic acid.   It reacts with caustic alkalies, but not with carbonates, and forms salts which are obtained by evaporating the solution, e.g. potassium phenate, C_6H_5OK.   These salts are stable to water, but are decomposed by carbon dioxide.

As an alcohol it will form esters, but owing to its acid character

the esters are not easily formed by the direct action of the acid.   They
are prepared by the action of the acid chloride, or anhydride, upon the
phenol, or its potassium salt :—

$$\text{C}_6\text{H}_5\text{OH} + \text{CH}_3\text{COCl} = \text{C}_6\text{H}_5\text{O.OC.CH}_3 + \text{HCl.}$$
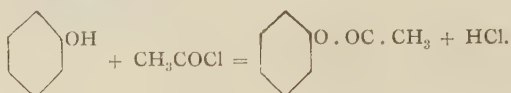
Phenyl sulphuric acid is present in mammalian urine.

Phenol also forms ethers ; these are prepared by the action of an
alkyl iodide upon potassium phenate :—

$$\text{C}_6\text{H}_5\text{OK} + \text{CH}_3\text{I} = \text{C}_6\text{H}_5\text{OCH}_3 + \text{KI.}$$
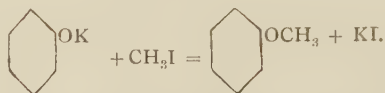
These ethers resemble the aliphatic ethers, but also show the
typical aromatic reactions with nitric acid, etc.

Reactions and Tests.

(1) A violet coloration is formed on adding a few drops of ferric
chloride solution to a solution of phenol in water.

(2) Phenol is readily brominated.   On adding bromine water
gradually to some phenol solution, there is first a cloudiness due to
mono- and dibromophenol which are characterised by a very penetrating
smell.   The further addition of bromine water produces a precipitate
of tribromophenol in yellowish-white needles, or flakes.   Tribromo-
phenol is formed directly with very dilute solutions of phenol.

(3) Phenol is also readily nitrated.   On adding concentrated nitric
acid to a solution of phenol and warming, a yellow colour is produced.
On cooling and making alkaline with ammonia, the colour becomes
orange.   Picric acid is formed :—

$$\text{C}_6\text{H}_5\text{OH} + 3\text{HNO}_3 = \text{C}_6\text{H}_2(\text{NO}_2)_3\text{OH} + 3\text{H}_2\text{O.}$$

(4) A deep red coloration is produced on adding Millon's reagent
to a solution of phenol and warming.

Detection of Phenol in Urine (Roaf).

1 c.c. of concentrated hydrochloric acid is added to 10 c.c. of horse's
urine and the mixture is boiled for two minutes.   The phenyl sulphuric
acid is hydrolysed.   The solution is cooled and extracted with ether.
The ethereal layer is separated and the ether evaporated.   The residue
dissolved in water will give the reaction with Millon's reagent.

It is better to distil the urine with dilute sulphuric acid—sufficient
concentrated acid being added to make the mixture contain 5 per cent.

—and collect about a quarter of the volume. The phenol can be tested for in the distillate.

Aniline, or Aminobenzene, or Phenylamine, $C_6H_5 . NH_2$.

Only small quantities of aniline are present in coal tar. It is usually obtained from nitrobenzene.

Nitrobenzene is converted into aniline by the action of reducing agents in acid solution :—

$$\text{(benzene ring)} NO_2 + 3H_2 = \text{(benzene ring)} NH_2 + 2H_2O.$$
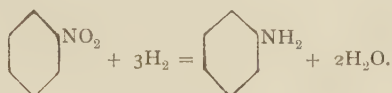
Preparation.

To 3 or 4 drops of nitrobenzene about 4 gm. of granulated tin and 3-4 c.c. of concentrated hydrochloric acid are added. The mixture is warmed to start the reaction and it is kept warm until the reaction ceases and until the smell of nitrobenzene is no longer perceptible. An excess of caustic soda is added and the alkaline solution is extracted with ether. The ether is allowed to evaporate in a basin and the residue is tested for aniline by treating it with bleaching powder solution ; a purple colour appears which becomes dirty red.

On a larger scale, 20 gm. of nitrobenzene and 40 gm. of granulated tin are placed in a litre flask and warmed on a water-bath for a few minutes. The flask is removed from the bath and fitted with an air condenser. 80 c.c. of concentrated hydrochloric acid are added in portions of 5 c.c. during the course of half an hour. If the mixture react violently, it is cooled in water. The reaction is completed by heating the flask on a boiling water-bath for about one hour. If the smell of nitrobenzene be still observed, more hydrochloric acid may be added and the heating continued until it vanishes. The double salt $(C_6H_5 . NH_2 . HCl)_2 . SnCl_4$ separates out if the product be allowed to cool ; it is diluted with 100 c.c. of water and immediately decomposed by carefully adding 65 gm. of caustic soda dissolved in 100 c.c. of water. Heat is developed on neutralising and stannic hydrate is precipitated ; this dissolves in excess of caustic soda and there results a dirty liquid containing aniline floating on the surface. The aniline is separated by distillation in steam (p. 10). The distillate is collected so long as drops of aniline pass over. The aniline is extracted by shaking it with ether and the ethereal solution is dried with solid caustic soda. The ether is distilled off from a water-bath and the residue is distilled over a flame. Aniline passes over at 182-184° as a pale yellow liquid.

Properties.

Aniline is a pale yellow oily liquid which boils at 182° and has a peculiar odour. It gradually turns brown on exposure to light and air.

Aniline is soluble with difficulty in water, but easily in alcohol and ether.

*   The solubility of aniline in water can be readily seen by placing 3 or 4 drops in a test tube full of water and shaking vigorously. The oily drops will be no longer visible.

Aniline is a weak base and forms salts with acids from which it is liberated by alkalies, thus :—

*   About 1 c.c. of aniline is placed in about 10 c.c. of dilute hydro-chloric acid. On shaking the aniline dissolves. On making alkaline with about 10 c.c. of caustic soda the aniline separates in oily drops :—

$$C_6H_5NH_2 + HCl = C_6H_5NH_2HCl$$
$$C_6H_5NH_2 . HCl + NaOH = C_6H_5NH_2 + H_2O + NaCl.$$
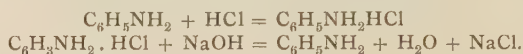
The salt of the aniline is obtained by evaporating its solution in the corresponding amount of acid until it crystallises :—

$$2C_6H_5NH_2 + H_2SO_4 = (C_6H_5NH_2)_2H_2SO_4.$$
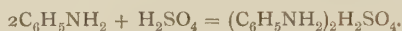
Reactions.

*   A solution of aniline in water is prepared as above.

(1) Aniline is readily brominated—bromine water is added to some of the solution ; a pinkish precipitate, which becomes grey-green, of tribromaniline is formed :—

$$C_6H_5NH_2 + 3Br_2 = C_6H_2Br_3 . NH_2 + 3HBr.$$

*   (2) On pouring some of the aqueous solution into a solution of bleach-ing powder, there is a violet coloration, which turns brown and fades.

*   (3) It turns black when it is oxidised with potassium bichromate and dilute sulphuric acid.

*   (4) It gives the carbylamine reaction with chloroform and alcoholic potash (p. 111).

*   (5) A blue colour is formed if a drop of aniline be mixed with 2 or 3 drops of strong sulphuric acid and the paste so formed stirred with a few drops of potassium bichromate solution.

Acetanilide, $C_6H_5 . NH . OC . CH_3$.

Aniline is acylated by treatment with acetyl chloride, glacial acetic acid, or acetic anhydride :—

$$C_6H_5 . NH_2 + HOOC . CH_3 = H_2O + C_6H_5 . NH—OC . CH_3.$$
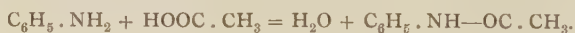
Preparation.

*   6 c.c. of aniline are boiled under a reflux air condenser with 12 c.c. of glacial acetic acid for an hour. The mixture is poured into water. Acetanilide is precipitated and is recrystallised from boiling water.

Properties.

Acetanilide is a white crystalline solid which melts at 114° and is used in medicine under the name of antifebrin.   It is readily decomposed by boiling with acids, or alkalies :—

$$C_6H_5NH—OC.CH_3 + H_2O = C_6H_5.NH_2 + HOOC.CH_3.$$

Thus, on boiling about 2 gm. of acetanilide with about 5 c.c. of concentrated hydrochloric acid for a few minutes and pouring the solution into water, a clear solution is obtained.   On adding excess of caustic soda, the aniline is precipitated and may be extracted with ether and tested for as above.

Alkyl Anilines.

Aniline as a primary amine will react with one or two molecules of an alkyl halide to form alkyl anilines :—

$$C_6H_5.NH_2 + CH_3I = C_6H_5.NH.CH_3 + HI$$
$$C_6H_5.NH.CH_3 + CH_3I = C_6H_5.N.(CH_3)_2 + HI.$$
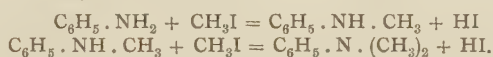
These compounds are readily obtained by heating aniline with the alcohol and hydrochloric acid at 200-250° :—

$$C_6H_5NH_2.HCl + CH_3OH = C_6H_5NH.CH_3HCl + H_2O$$
$$C_6H_5NH_2.HCl + 2CH_3OH = C_6H_5.N(CH_3)_2HCl + 2H_2O.$$
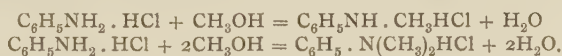
The methyl anilines are stronger bases than aniline, as they are more like the aliphatic amines.   They may be regarded as phenylmethylamine and phenyldimethylamine.

Methyl aniline is a colourless oily liquid which boils at 192°.   As a secondary amine it gives a nitrosamine with nitrous acid :—

$$\begin{matrix} C_6H_5 \\ \diagdown \\ \diagup \\ CH_3 \end{matrix} NH + O:N.OH = \begin{matrix} C_6H_5 \\ \diagdown \\ \diagup \\ CH_3 \end{matrix} N.NO + H_2O.$$
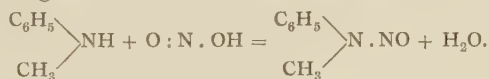
Phenyl-methyl-nitrosamine is a yellow oil which gives Liebermann's nitroso reaction.

Dimethylaniline is a colourless oil which boils at 192°; it is largely used in the dye industry.

Dialkylanilines, such as dimethylaniline, react with nitrous acid giving nitroso compounds : in these compounds the reaction takes place with the hydrogen atom in the para position (p. 178) in the benzene nucleus :—

$$C_6H_5.N(CH_3)_2 + ONOH = C_6H_4 \begin{matrix} \diagup NO \\ \diagdown N(CH_3)_2 \end{matrix} + H_2O.$$
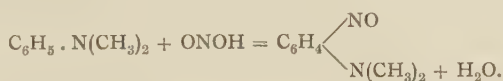
Diphenylamine cannot be prepared by heating aniline with bromobenzene, but is obtained by heating aniline hydrochloride with aniline in a closed vessel at 240° :—

$$C_6H_5NH_2.HCl + C_6H_5NH_2 = \begin{matrix} C_6H_5 \\ \diagdown \\ \diagup \\ C_6H_5 \end{matrix} NH + NH_4Cl.$$
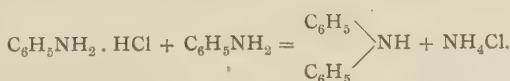
Diphenylamine is a crystalline solid melting at 54° and boiling at 310°. It is a very weak base, its salts being decomposed by water; it is almost

insoluble in dilute acids. It dissolves in concentrated sulphuric acid. This solution on the addition of a trace of nitric acid gives a deep blue coloration and serves for detecting nitrates. Diphenylamine owing to the acid character of the phenyl groups reacts with potassium giving potassium diphenylamine,

$$\begin{matrix} C_6H_5 \\ \\ C_6H_5 \end{matrix}\Big\rangle NK.$$
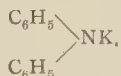
Triphenylamine, $(C_6H_5)_3N$.

This compound is prepared by heating potassium diphenylamine with bromobenzene at 300°. It is a colourless crystalline solid melting at 127°. It does not form salts with acids.

Diazobenzene, $C_6H_5 . N : N . OH$, or $C_6H_5 . N(_1 N) . OH$.

Diazonium Salts, e.g., $C_6H_5N_2 . Cl$.

Aniline is a primary amine. Primary amines of the aliphatic series are converted by the action of nitrous acid into the corresponding alcohol. In the aromatic series an intermediate compound, known as the diazo compound, is first formed. It is decomposed on warming with evolution of nitrogen :—

$$C_6H_5NH_2 + ONOH = C_6H_5N_2OH + H_2O$$
$$C_6H_5N_2OH = C_6H_5OH + N_2.$$
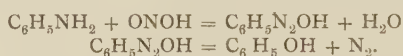
The reaction is carried out by dissolving aniline in two equivalents of hydrochloric acid and adding slowly 1 molecule of sodium nitrite to the solution kept at about 0°. The presence of excess of sodium nitrite is shown by testing with starch-iodide paper.

In this reaction the salt, diazobenzene chloride, or diazonium chloride, is formed. The salt can be isolated by carrying out the reaction in alcoholic solution. The isolation of the salt is not necessary for carrying out the various reactions of diazobenzene.

Properties.

Diazobenzene behaves like a strong base (NH_4OH) and is known *only* in the form of its salts. When liberated from a solution of its salts, it is precipitated as a yellow oil which is very unstable and decomposes with explosion.

Its crystalline salts with mineral acids are also explosive. The nitrate explodes violently if gently struck ; the other salts explode on heating. These salts are easily soluble in water, less soluble in alcohol and insoluble in ether. They are generally called diazonium salts.

Diazobenzene and the diazonium salts are usually represented by the formula proposed by Blomstrand :—
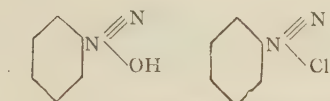
Diazobenzene.   Diazonium chloride.

The diazo compounds have numerous reactions and serve for the preparation of other derivatives of benzene, thus :—

Reactions.

(1) An aqueous solution of a diazonium salt is decomposed on boiling.   Nitrogen is evolved and phenol is formed :—

$$C_6H_5 . N_2Cl + H_2O = C_6H_5OH + N_2 + HCl.$$

Thus :—

A few drops of sodium nitrite solution are added to a dilute solution of aniline in hydrochloric acid.   Diazobenzene chloride is formed.   On warming the solution, it is decomposed with evolution of nitrogen and the smell of phenol (carbolic acid) becomes noticeable.

(2) On boiling a diazobenzene salt with absolute alcohol, nitrogen is evolved and benzene is formed, reduction occurring :—

$$C_6H_5N_2Cl + H_2 = C_6H_6 + N_2 + HCl.$$
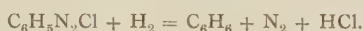
(3) A precipitate of diazobenzene perbromide is formed on adding bromine dissolved in potassium bromide to a solution of diazobenzene chloride; on boiling with alcohol, nitrogen is evolved and bromobenzene is formed :—

$$C_6H_5N_2Cl + Br_2 + KBr = KCl + C_6H_5NBr . NBr_2.$$
$$C_6H_5NBr . NBr_2 = C_6H_5Br + N_2 + Br_2.$$
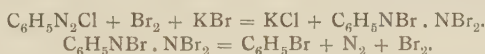
(4) Iodobenzene is formed if potassium iodide be added to a solution of diazobenzene chloride and the solution warmed :—

$$C_6H_5N_2Cl + KI = KCl + N_2 + C_6H_5I.$$
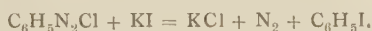
(5) *Sandmeyer's Reactions.*

On adding a solution of cuprous chloride in hydrochloric acid,
or   ,,   ,,   ,,   ,,   ,, cuprous bromide in hydrobromic acid,
or   ,,   ,,   ,,   ,,   ,, cuprous cyanide in potassium cyanide,
to a solution of diazobenzene chloride and warming, nitrogen is evolved and chlorobenzene, bromobenzene or cyanobenzene (phenyl-cyanide) is formed :—

$$C_6H_5N_2Cl + CuCN = C_6H_5CN + N_2 + CuCl.$$
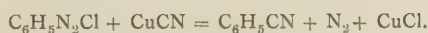
In practice, these reactions are carried out by mixing aniline with a slight excess of hydrochloric acid, cooling in ice and adding the calculated quantity of sodium nitrite solution, as shown by testing with potassium iodide-starch

paper.   The aqueous solution is warmed, or potassium iodide is added, or it is poured into the solution of the cuprous salt and warmed.   The product can generally be isolated by steam distillation.

Phenylhydrazine, $C_6H_5 . NH . NH_2$.

Phenylhydrazine is obtained by reducing diazonium chloride with stannous chloride :—

$$C_6H_5 . N_2 . Cl + 2H_2 = C_6H_5NH . NH_2 . HCl.$$

Its constitution is proved by its conversion into aniline and ammonia by reduction with zinc and hydrochloric acid :—

$$C_6H_5NH . NH_2 + H_2 = C_6 H_5NH_2 + NH_3.$$

Preparation.

A molecular proportion of aniline (9·3 gm.) is dissolved in about 10 times the calculated quantity of concentrated hydrochloric acid (200 c.c.), thoroughly cooled in ice and diazotised by adding the calculated quantity of sodium nitrite (6·9 gm.).   As soon as excess of nitrite is present, as indicated by starch-iodide paper, rather more than the calculated quantity of stannous chloride (45 gm.) dissolved in the proper amount of concentrated hydrochloric acid (100 c.c.) is slowly added.   Phenylhydrazine hydrochloride separates out. It is filtered off by suction and washed with concentrated hydrochloric acid. It is dissolved in water and decomposed with excess of caustic soda ; the oil is extracted with ether, the ethereal solution dried with solid potash, the ether distilled off and the base distilled *in vacuo*.

Properties.

Phenylhydrazine consists of colourless prisms which melt at 23° and boil at 241° with slight decomposition.   It dissolves slightly in cold water and easily in alcohol and ether.   As a strong base, it forms salts with acids ;   the hydrochloride crystallises in needles and is easily soluble in warm water.

Reactions.

(1) Phenylhydrazine and its salts reduce Fehling's solution.

(2) It is converted into benzene on heating its solutions with copper sulphate, or ferric chloride.

(3) It combines with aldehydes, ketones, and carbohydrates to form hydrazones and osazones.   The hydrazone is decomposed by concentrated hydrochloric acid, and on reduction gives an amine and aniline.

Toluene, $C_6H_5 . CH_3$.

Toluene, or methylbenzene, or phenylmethane, is present in coal tar and is contained with benzene in the first fraction on fractionally distilling the tar.   It is separated from benzene by fractional distillation.

Toluene is also obtained from balsam of tolu, or from toluic acid by

distillation with soda lime—a reaction analogous to the preparation of methane from sodium acetate :—

$$C_6H_4\Big\langle{}^{CH_3}_{COOH} = C_6H_5 . CH_3 + CO_2.$$
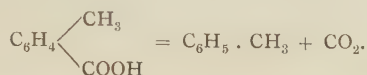
Toluene can be prepared from benzene by either of the following two reactions :—

(1) *Fittig's Reaction.*—A mixture of bromobenzene and methyl bromide is heated with sodium :—

$$C_6H_5Br + Na_2 + CH_3Br = C_6H_5 . CH_3 + 2NaBr.$$
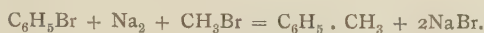
(2) *Friedel and Craft's Reaction.*—Benzene is heated with methyl iodide in the presence of aluminium chloride :—

$$C_6H_6 + CH_3I = C_6H_5 . CH_3 + HI.$$

In this reaction a compound of benzene and aluminium chloride is probably first formed and this compound reacts with the alkyl halide :—

$$C_6H_6 + Al_2Cl_6 = C_6H_5 . Al_2Cl_5 + HCl$$
$$C_6H_5Al_2Cl_5 + CH_3Cl = Al_2Cl_6 + C_6H_5 . CH_3.$$
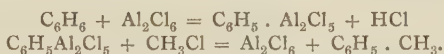
Dry benzene is treated under a reflux condenser with a third of its weight of aluminium chloride and the alkyl chloride is slowly added. The benzene may be mixed with a neutral solvent such as ether, or petroleum ether. The mixture is heated on a water-bath until halogen acid is no longer evolved. The mixture is allowed to cool and water added to dissolve the aluminium chloride; the layer of benzene and ether is separated, dried with calcium chloride, the ether distilled off and the residue distilled.

Properties.

Toluene, an oily colourless liquid with characteristic smell, boils at 110° and has a sp. gr. of ·882 at 0°. It burns with a smoky luminous flame, is insoluble in water, but soluble in organic solvents. It is known commercially as toluole.

Toluene closely resembles benzene in its properties in forming nitro- and other derivatives, but it differs from benzene in being also an aliphatic compound. Toluene is the first instance of an aromatic compound containing a side chain. It is this side chain which gives toluene the properties of an aliphatic compound as well as an aromatic compound. (See benzyl chloride, benzyl alcohol, benzaldehyde, etc.)

On oxidation with dilute nitric acid and other oxidising agents, the nucleus remains intact but the side chain is oxidised to a carboxyl group :—

$$C_6H_5 . CH_3 + 3O = C_6H_5 . COOH + H_2O.$$
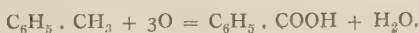
Ethylbenzene, $C_6H_5 . C_2H_5$.

Ethylbenzene is also contained in coal tar and can be prepared from benzene by the reactions given under toluene. It is a liquid like toluene, but boils at $134°$ and is isomeric with the xylenes (p. 203).

Other Homologues of Benzene.

The other homologues, propyl, butyl, etc., benzene can be prepared in the same way as toluene from alkylhalide and benzene, or bromo-benzene.

All the homologues of benzene contain both a benzene nucleus and an aliphatic radicle, or side chain. They behave as aromatic compounds by forming nitro and sulphonic acid derivatives. They behave as aliphatic compounds in forming halogen derivatives in which the halogen atom can be replaced by OH and other groups. On oxidation, the side chain undergoes shortening until ultimately benzoic acid is formed :—

$$C_6H_5 . CH_2 . CH_2 . CH_3 \rightarrow C_6H_5 . CH_2 . CH_2 . COOH \rightarrow C_6H_5 . CH_2 . COOH \rightarrow C_6H_5 . COOH.$$
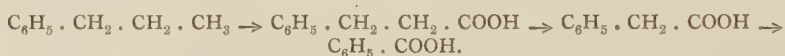
Styrene, or Phenylethylene, $C_6H_5 . CH = CH_2$, is an aromatic hydrocarbon containing an unsaturated group in the side chain. It is obtained by heating cinnamic acid.

Benzyl Chloride, $C_6H_5 . CH_2Cl$.

Benzyl chloride is the chief representative of an aromatic compound in which a halogen atom is present in the side chain. Like aliphatic compounds, it can be obtained by the action of phosphorus pentachloride upon the corresponding alcohol—benzyl alcohol $C_6H_5 . CH_2OH$. The radicle $C_6H_5 . CH_2$ is termed benzyl in order to distinguish it from the radicle C_6H_5, which is termed phenyl.

Preparation.

Benzyl chloride is prepared by passing a stream of dry chlorine into toluene, heated under a reflux condenser, until the increase in weight corresponding to the equation has been reached. The product is then separated and purified by distillation. The reaction takes place most readily, if the flask be exposed to sunlight. The following reaction takes place :—

$$C_6H_5 . CH_3 + Cl_2 = C_6H_5 . CH_2Cl + HCl.$$
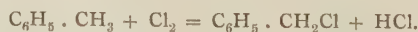
The procedure is quite different to that used in the preparation of bromobenzene and chlorotoluene (p. 182).

Properties.

Benzyl chloride is a colourless liquid which boils at 176°. It has an unpleasant smell, is insoluble in water, but soluble in alcohol, ether, and benzene.

It is nitrated, sulphonated, etc., by nitric or sulphuric acid, but in its other reactions it resembles ethyl chloride. It is mainly used for the preparation of benzaldehyde.

Benzal Chloride, $C_6H_5 . CHCl_2$.

This compound is prepared by the further action of chlorine upon boiling toluene, until chlorine corresponding to the equation,

$$C_6H_5 . CH_3 + 2Cl_2 = C_6H_5CHCl_2 + 2HCl,$$
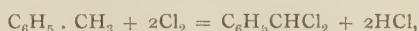
has been absorbed.

It can be obtained by the action of phosphorus pentachloride upon benzaldehyde :—

$$C_6H_5 . CHO + PCl_5 = C_6H_5CHCl_2 + POCl_3.$$

Benzal chloride is a colourless liquid of boiling-point 206° ; it is also used for making benzaldehyde.

Benzotrichloride, $C_6H_5 . CCl_3$, or Phenylchloroform.

By the further action of chlorine upon toluene, benzotrichloride is formed :—

$$C_6H_5CH_3 + 3Cl_2 = C_6H_5CCl_3 + 3HCl.$$
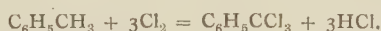
It is a liquid which boils at 213° and is converted into benzoic acid by boiling with water.

Benzyl Alcohol, $C_6H_5 . CH_2OH$.

Benzyl alcohol occurs as such and also as ester with benzoic and cinnamic acids in the resin storax, in balsam of Tolu and balsam of Peru.

It is the chief type of an aromatic alcohol in which the hydroxyl group is present in the side chain (compare phenol).

Preparation.

As an alcohol, it may be obtained by reducing the corresponding aldehyde, benzaldehyde :—

$$C_6H_5 . CHO + H_2 = C_6H_5 . CH_2OH,$$
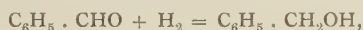
or by the action of water and aqueous alkalies upon benzyl chloride :—

$$C_6H_5CH_2Cl + H_2O = HCl + C_6H_5 . CH_2OH.$$
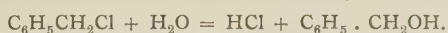
Benzyl alcohol is usually obtained by the action of aqueous potassium hydroxide upon benzaldehyde :—

$$2C_6H_5CHO + KOH = C_6H_5CH_2OH + C_6H_5COOK,$$

Benzaldehyde is shaken up with four times the amount of potash dissolved in about an equal weight of water. The emulsion which is formed is allowed to stand for twenty-four hours. On the addition of water, the potassium benzoate dissolves ; the solution is extracted with ether, the ether dried, and the benzyl alcohol obtained by distillation.

Properties.

Benzyl alcohol is a colourless liquid of boiling-point 206° ; it is not easily soluble in water, but dissolves in alcohol and ether. It behaves like ethyl alcohol with sodium and phosphorus pentachloride. It forms esters with acids, or acid anhydrides, etc., e.g. benzyl bromide, benzyl acetate.

Benzaldehyde, $C_6H_5 . CHO$.

Benzaldehyde was originally isolated from bitter almonds and called oil of bitter almonds. The almonds contain the glucoside, amygdalin, which is hydrolysed by the enzyme, emulsin, into glucose, benzaldehyde and prussic acid. It is the aldehyde of benzyl alcohol from which it may be obtained by oxidation with nitric acid.

It may be obtained by distilling calcium benzoate with calcium formate in the same way as aliphatic aldehydes.

Preparation.

(1) Benzaldehyde is prepared from benzal chloride by boiling it with dilute sulphuric acid, or lime water, under pressure :—

$$C_6H_5 . CHCl_2 \rightarrow C_6H_5CH(OH)_2 \rightarrow C_6H_5 . CHO.$$

(2) It is prepared by boiling benzyl chloride with lead nitrate, or copper nitrate. Benzyl alcohol is probably first formed and is oxidised to the aldehyde :—

$$C_6H_5 . CH_2Cl \rightarrow C_6H_5CH_2OH \rightarrow C_6H_5 . CHO.$$

Molecular proportions of benzyl chloride (12·6 gm.) and copper nitrate (20 gm.), dissolved in about the same weight of water (25 c.c.), are boiled for 6-8 hours under a reflux condenser, whilst a current of carbon dioxide is passed through the mixture to expel oxides of nitrogen and to avoid further oxidation. When the oil contains no chlorine, or only traces, as shown by testing it, after washing with water, with silver nitrate and nitric acid, the oil is extracted with ether and the ethereal extract is shaken with saturated sodium bisulphite solution. The crystalline bisulphite compound is filtered off and washed with ether. The benzaldehyde is obtained by decomposing it with dilute sulphuric acid, extracting with ether, drying, and distilling.

(3) It is prepared by Friedel and Craft's reaction from benzene, a mixture of carbon monoxide and chlorine being passed into the benzene. Formyl-chloride is apparently formed which reacts as follows :—

$$C_6H_6 + H . CO . Cl = C_6H_5 . CHO + HCl.$$

Properties.

Benzaldehyde is a colourless liquid with a strong smell of bitter almonds.   It boils at 179° and has a sp. gr. of 1·05 at 15°.   It is very slightly soluble in water, but dissolves in alcohol and ether.   It is used extensively for flavouring purposes.

Reactions.

In most reactions benzaldehyde resembles the aliphatic aldehydes :—

* (1) It is easily oxidised ; by exposure to air, crystals of benzoic acid gradually separate :—

$$C_6H_5 . CHO + O = C_6H_5 . COOH.$$
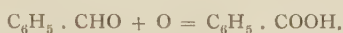
(2) On reduction, it yields benzyl alcohol.

(3) It yields benzal chloride with phosphorus pentachloride.

(4) It gives an oxime with hydroxylamine.

* (5) It gives a hydrazone with phenylhydrazine.

* (6) It combines with sodium bisulphite.

(7) It combines with hydrogen cyanide.

In the following reactions benzaldehyde and other aromatic aldehydes, which have the aldehyde group attached to the benzene nucleus, differ from aliphatic aldehydes :—

* (1) It does not reduce Fehling's solution, or ammoniacal silver solutions.

(2) It does not polymerise.

(3) It gives a mixture of alcohol and acid on treating with potash.

(4) It is converted into benzoin on shaking with an alcoholic solution of potassium cyanide :—

$$C_6H_5 . CHO + C_6H_5 . CHO = C_6H_5 . CO . CHOH . C_6H_5.$$
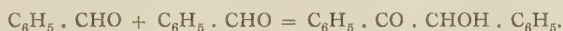
Benzoin is a complex ketonic alcohol formed by the condensation of two molecules of benzaldehyde.

As an aromatic compound benzaldehyde forms nitro, sulphonic acid derivatives, etc.

Benzoic Acid, $C_6H_5 . COOH.$

Benzoic acid occurs in gum benzoin and other resins such as balsam of Peru.   In gum benzoin it is present chiefly as the ester, benzyl benzoate.

Preparation.

(1) Benzoic acid is readily obtained by subliming gum benzoin. Gum benzoin is heated on an iron tray, or porcelain basin, the tray being covered with a cone of filter paper, or a funnel.   The resin melts

and the benzoic acid which volatilises condenses on the cone.   It is recrystallised from water.

(2) Benzoic acid is made commercially by oxidising benzyl chloride with 60 per cent. nitric acid.

(3) It is also prepared by heating the calcium salt of phthalic acid.

(4) It can be prepared by the hydrolysis of the nitrile, phenyl cyanide.

(5) As previously mentioned, it results from the oxidation of aromatic compounds possessing a side chain.

Properties.

Benzoic acid forms glistening crystals which melt at $121.5°$ and boil at $249°$.

*   On heating, it melts and gives off white vapours with characteristic smell and suffocating effect upon the throat ; the vapours condense as a crystalline sublimate.

It dissolves easily in hot water and crystallises out on cooling ; it is only slightly soluble in cold water (1 part in 400).

It dissolves in alcohol and ether and other organic solvents.

*   It forms salts with alkalies, dissolving in caustic alkalies and alkali carbonates, in lime water, etc. ; on acidifying these solutions, it is precipitated.

*   A neutral solution gives a precipitate of a pale brown colour with ferric chloride.

*   It is easily nitrated : nitrobenzoic acid is formed on evaporating a little benzoic acid in a porcelain basin with nitric acid.

It is converted into benzene by heating with soda lime.

It forms esters, e.g. ethylbenzoate (p. 68) is formed if benzoic acid be heated with a little alcohol and a few drops of concentrated sulphuric acid.   The ester has a peculiar aromatic odour and boils at $213°$.

Benzoyl Chloride, $C_6H_5 . CO . Cl$.

This compound is formed by the action of phosphorus pentachloride upon benzoic acid :—

$$C_6H_5 . COOH + PCl_5 = C_6H_5COCl + POCl_3 + HCl.$$

Benzoic acid and a slight excess of phosphorus pentachloride are placed in a distilling flask ; the reaction proceeds at the ordinary temperature and the vapours of hydrogen chloride are passed into soda.   As soon as the reaction is complete, the contents are distilled in a fume cupboard ; phosphorus oxychloride passes over at $107°$, benzoyl chloride at about $198°$.   It is purified by redistillation.

Benzoyl chloride is a colourless oily liquid with a peculiar pungent smell.   It is slowly decomposed by water into benzoic acid and more readily by alcohol into ethyl benzoate.

Benzoic Anhydride, $(C_6H_5 . CO)_2O$.

The anhydride of benzoic acid is prepared, like other anhydrides, by the action of benzoyl chloride upon sodium benzoate :—

$$C_6H_5COCl + C_6H_5COONa = C_6H_5 . CO . O . CO . C_6H_5 + NaCl.$$

It is a colourless crystalline substance melting at $42°$ and resembles acetic anhydride.

Benzamide, $C_6H_5 . CO . NH_2$.

Benzamide is prepared by either of the reactions :—
(1) Benzoyl chloride and ammonia :—

$$C_6H_5COCl + NH_3 = C_6H_5 . CO . NH_2 + HCl.$$

Benzoyl chloride is mixed with a slight excess of dry ammonium carbonate in a mortar until the smell of benzoyl chloride vanishes.   Cold water is added to dissolve the ammonium salts ; the insoluble benzamide is crystallised from hot water.

(2) Ammonia and ethyl benzoate.
Benzamide is a colourless crystalline solid melting at $130°$, easily soluble in hot water, but soluble with difficulty in cold.   It is decomposed by boiling with acids, or alkalies, into benzoic acid and ammonia.

Benzonitrile, or Phenyl Cyanide, $C_6H_5 . CN$.

Benzonitrile is prepared :—
(1) by fusing potassium benzene sulphonate with potassium cyanide, or ferrocyanide :—

$$C_6H_5SO_3K + KCN = C_6H_5CN + K_2SO_3 ;$$

(2) by Sandmeyer's reaction from aniline ; the aniline is diazotised and the solution heated with cuprous cyanide (p. 191).
Benzonitrile is a colourless oil with a smell resembling that of nitrobenzene.   It boils at $191°$ and resembles the aliphatic nitriles in its reactions :—
(1) hydrolysis :—

$$C_6H_5CN + 2H_2O = C_6H_5COOH + NH_3,$$

(2) reduction :—
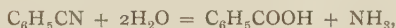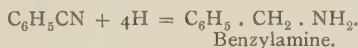
$$C_6H_5CN + 4H = C_6H_5 . CH_2 . NH_2.$$
Benzylamine.

Benzylamine, $C_6H_5 . CH_2 . NH_2$.

Benzylamine is an aromatic amine in which the amino group is present in the side chain (compare aniline).   It is prepared like the aliphatic primary amines (p. 109) :—
(1) ammonia upon benzyl chloride ;
(2) bromine and potash upon the amide of phenylacetic acid ;
(3) reduction of the nitrile, or oxime.
It is a colourless oily liquid boiling at $187°$ with pungent smell.   It is a strong base like the aliphatic primary amines and with similar properties.

Dibenzylamine, $(C_6H_5.CH_2)_2NH$.   Tribenzylamine, $(C_6H_5.CH_2)_3N$.

Again, these compounds resemble the secondary and tertiary aliphatic amines.   They are obtained by heating benzylamine with benzyl chloride.

The three amines are formed when benzyl chloride is heated with ammonia. They have the typical aromatic reactions, as well as the aliphatic ones.

Acetophenone, $C_6H_5 . CO . CH_3$.

Acetophenone is an example of an aromatic ketone.   It is formed by distilling calcium benzoate with calcium acetate.

Acetophenone is most readily prepared by slowly dropping 1 molecular proportion of acetyl chloride upon 1 molecule of benzene containing in suspension 1 molecule of aluminium chloride and cooled with ice.   When the evolution of hydrochloric acid is over, ice-cold water is very carefully added. The solution is extracted with ether, the ethereal extract dried and distilled. Acetophenone passes over between 194 and 200° :—

$$C_6H_6 + CH_3COCl = C_6H_5 . CO . CH_3 + HCl.$$

This reaction is a general one for preparing aromatic ketones.

Acetophenone is a crystalline solid melting at 20·5° and boiling at 202°. It is soluble in water and alcohol.   It is sometimes used as a hypnotic under the term hypnone.   It closely resembles acetone and the aliphatic ketones in giving a secondary alcohol on reduction, benzoic acid and acetic acid on oxidation, and in forming an oxime and a phenylhydrazone.   It resembles benzene in forming nitro- and other derivatives.

Benzophenone, $C_6H_5 . CO . C_6H_5$.

Benzophenone is obtained by heating calcium benzoate and is prepared by the action of benzoyl chloride upon benzene in the presence of aluminium chloride.

It is a crystalline solid melting at 48-49° and resembles acetophenone. It yields diphenylmethane on reduction with zinc dust.

Phenylacetic Acid, $C_6H_5 . CH_2 . COOH$.

This acid containing a carboxyl group in the side chain is formed in the putrefaction of proteins, arising from phenylalanine.

Synthetically it is obtained from benzyl chloride :—

$$C_6H_5 . CH_2Cl \rightarrow C_6H_5 . CH_2 . CN \rightarrow C_6H_5 . CH_2 . COOH.$$

Molecular proportions of benzyl chloride and potassium cyanide are boiled in dilute alcoholic solution for 3-4 hours.   The benzyl cyanide is isolated by fractional distillation (220-335° fraction) and hydrolysed by boiling with dilute sulphuric acid.   The phenylacetic acid is purified by crystallisation.

Phenylacetic acid crystallises in colourless shining plates, which melt at 76·5° and boil at 262°.   It has a characteristic smell.

Phenaceturic Acid, $C_6H_5 . CH_2 . CO—NH . CH_2 . COOH$.

Phenylacetic acid, which is formed in the large intestine in small amounts, on absorption into the body, or when it is injected into the blood, is excreted as phenaceturic acid, i.e. in combination with glycine.   It thus resembles benzoic acid, which is excreted as hippuric acid (p. 171).

It is a colourless crystalline substance melting at 143°, is soluble with

difficulty in water, but easily in alcohol. It is hydrolysed by boiling with acids into phenylacetic acid and glycine.

Phenylpropionic Acid, $C_6H_5 . CH_2 . CH_2 . COOH$.

Phenyl propionic acid accompanies phenylacetic acid amongst the putrefaction products of proteins.

It is most conveniently prepared by reducing cinnamic acid :—

$$C_6H_5 . CH{=}CH . COOH + H_2 = C_6H_5 . CH_2 . CH_2 . COOH.$$

It is a colourless crystalline substance melting at 47° and boiling at 280°.

Cinnamic Acid, $C_6H_5 . CH{=}CH . COOH$.

Cinnamic acid is the chief representative of an aromatic compound containing an unsaturated acid as the side chain. It occurs in greatest amount in storax, the resin of *Styrax officinalis.*

It is prepared from storax by warming it with dilute sodium hydroxide ; the filtered alkaline solution is acidified with hydrochloric acid. Cinnamic acid is precipitated and purified by crystallisation from water.

Cinnamic acid is usually prepared by synthesis by Perkin's reaction :—

$$C_6H_5 . CHO + H_3C . COONa = C_6H_5 . CH{=}CH . COONa + H_2O.$$

Molecular proportions of benzaldehyde and sodium acetate are heated in an oil-bath under a reflux condenser with 3-4 parts of acetic anhydride for 8-10 hours. The mixture is poured into water and unchanged benzaldehyde is removed by steam distillation; the residue is treated with caustic soda, filtered from oily impurities and acidified with concentrated hydrochloric acid. Cinnamic acid is precipitated and purified by crystallisation from water.

Cinnamic acid crystallises in needles and melts at 133°. It is soluble with difficulty in cold water, more easily in hot water. It dissolves in alcohol, ether, and other organic solvents. As an aromatic compound, it forms nitro derivatives. As an unsaturated aliphatic compound, cinnamic acid forms addition compounds with bromine and halogen acids. It is converted by reduction into phenylpropionic acid, or hydrocinnamic acid. It is readily oxidised in the cold by permanganate, a solution of cinnamic acid in alkali decolorising permanganate immediately.

Cinnamic Aldehyde, $C_6H_5 . CH{=}CH . CHO$.

Cinnamic aldehyde is the chief constituent of oil of cinnamon from which it may be obtained by treatment with sodium bisulphite.

It is a liquid which boils at 247° and has the peculiar and characteristic odour of cinnamon. It resembles the aliphatic aldehydes in properties.

Phenylalanine, $C_6H_5 . CH_2 . CH . (NH_2)COOH$.

Phenylalanine was first isolated from an extract of growing seedlings and subsequently recognised as a constituent of proteins ; it is the constituent which gives rise to phenylpropionic and phenylacetic acids during putrefaction. It also gives rise to phenylethylamine, and most probably cinnamic acid is also derived from it.   In the

decomposition either carbon dioxide, or ammonia, is lost and then the side chain is oxidised.:—

$$C_6H_5.CH_2.CH_2.COOH \leftarrow C_6H_5.CH_2.CH(NH_2)COOH \rightarrow C_6H_5.CH_2.CH_2.NH_2$$
$$\downarrow$$
$$C_6H_5.CH_2.COOH \rightarrow C_6H_5.COOH.$$
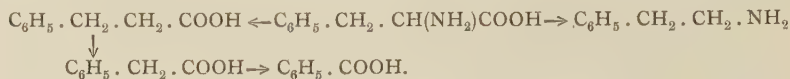
Phenylalanine is isolated with some difficulty from the complicated mixture of amino acids resulting from proteins. It is more easily prepared by synthesis.

Phenylalanine crystallises in glistening platelets which melt at 275-280°. The natural substance is laevorotatory. It has aromatic properties and the properties of an amino acid.

Mandelic Acid, $C_6H_5.CHOH.COOH.$

Mandelic acid is formed when amygdalin is hydrolysed by boiling with acids; it is present in amygdalin as the nitrile of mandelic acid:—

$$C_6H_5.CH\begin{cases} O.C_{12}H_{22}O_{11} \\ CN \end{cases} + 2H_2O = C_6H_5.CH\begin{cases} OH \\ CN \end{cases} + 2C_6H_{12}O_6$$

$$C_6H_5.CH\begin{cases} OH \\ CN \end{cases} + 2H_2O = C_6H_5.CHOH.COOH + NH_3.$$
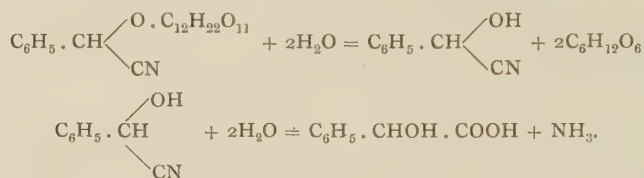
It is prepared synthetically from benzaldehyde; the benzaldehyde is converted into the cyanohydrin and this is hydrolysed.

Mandelic acid is a colourless crystalline solid which melts at 133°. It is soluble in water, alcohol, ether, and other organic solvents. The natural acid is optically active and laevorotatory; the synthetical acid has been separated into its stereisomers by the usual methods.

Mandelic acid closely resembles lactic acid in its properties, as it contains the OH group in the side chain. It differs from salicylic acid and other aromatic acids which contain the OH group attached to the benzene nucleus.

CHAPTER XXIII.

DISUBSTITUTION DERIVATIVES OF BENZENE.

THE disubstitution products of benzene exist in three forms :—

ortho   meta   para
o.   m.   p.
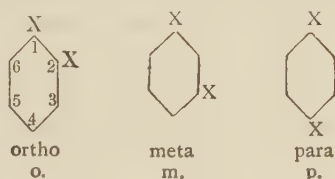
The number of derivatives is very large. They can be divided into those in which the substituting groups are the same and those in which they are different.

They are produced in general by the action of the various reagents such as nitric and sulphuric acids upon the mono-substituted derivative of benzene, and then reducing the nitro compound to form the amine, or fusing the sulphuric acid compound with potash to obtain the phenol. The particular isomer which is formed depends upon the nature of the group already attached to the benzene ring. These groups exert a sort of directing influence upon the position taken up by the entering radicle. Thus, if the radicle already present is NO_2, SO_3H, $COOH$, i.e. of acid character, the entering NO_2, or SO_3H, group takes up the meta position. If the radicle already present is CH_3, OH, NH_2, Cl, the entering group takes the para position and also the ortho position.

If therefore benzoic acid be nitrated, meta-nitro-benzoic acid is preferentially formed. Toluene on nitration gives a mixture of o- and p-nitrotoluenes, which on oxidation will give o- and p-nitro-benzoic acids. Again, nitration of chlorobenzene gives o- and p-chloro-nitro-benzene and chlorination of nitrobenzene gives m-chloro-nitrobenzene.

Dimethylbenzenes, or Xylenes, $C_6H_4\diagdown\genfrac{}{}{0pt}{}{CH_3}{CH_3}$.
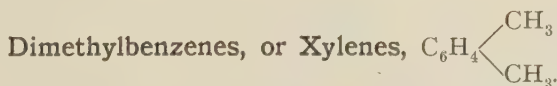
These three compounds are present in coal tar and are contained in the benzene fraction from which they are prepared by fractional distillation; m-xylene exists in largest amount. The fraction in which they are present boils at 136-141°. Their boiling-points are so close that they cannot be

separated by fractional distillation ; their separation depends on the formation of nitro- and sulphonic acid derivatives.

They closely resemble benzene and toluene and are obtained from these compounds by the same methods as toluene is prepared from benzene. A different isomer is formed under different conditions.

They yield nitro- and other derivatives, and on oxidation are converted into methyl benzoic acids and into phthalic acids.

$$\text{Dinitrobenzenes, } C_6H_4\diagup^{NO_2}_{\diagdown NO_2.}$$
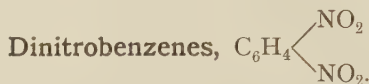
m-Dinitrobenzene is obtained by nitrating benzene with sulphuric acid and nitric acid and heating. It is a yellow crystalline solid melting at 90°.

o- and p-Dinitrobenzenes are formed in small quantities at the same time. They are colourless solids melting at 118° and 173° respectively.

$$\text{Diaminobenzenes, or Phenylenediamines, } C_6H_4\diagup^{NH_2}_{\diagdown NH_2.}$$
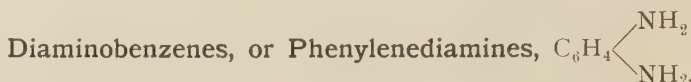
m-Phenylenediamine is obtained by reducing m-dinitrobenzene. The o- and p-phenylenediamines are prepared by nitrating acetanilide and subsequently removing the acetyl group by hydrolysis and reducing the nitrogroup. They resemble aniline.

m-Phenylenediamine melts at 63°. It gives a deep yellow colour with nitrites and is used for detecting nitrites in small quantities.

$$\text{Benzene Disulphonic Acids, } C_6H_4\diagup^{SO_3H}_{\diagdown SO_3H.}$$

The m-compound is formed by heating benzene with two molecular proportions of sulphuric acid.

$$\text{Dihydroxy-Benzenes, } C_6H_4\diagup^{OH}_{\diagdown OH.}$$
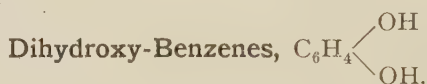
Two of the three isomeric dihydroxy benzenes are natural compounds. They are termed :—

Catechol,
or
pyrocatechin

Resorcinol

Hydroquinone,
or
quinol

Ortho.   Meta.   Para.

Catechol occurs in catechu, a resin obtained from *Acacia catechu*, and was first obtained from this source. It is prepared by fusing o-phenolsulphonic acid with potash, or by reducing guaiacol with hydriodic acid :—

$$C_6H_4\diagup^{OCH_3}_{\diagdown HO} + HI = CH_3I + C_6H_4\diagup^{OH}_{\diagdown OH.}$$

It is a colourless crystalline solid melting at 104°.

Resorcinol is obtained by fusing benzene-m-disulphonic acid and the other disulphonic acids with potash at higher temperatures.

It forms colourless crystals which melt at 110° and are easily soluble in water, alcohol, ether. It is used largely for making eosin and other dyes.

Quinol is formed by the hydrolysis of the glucoside, arbutin, by boiling with water. It is usually prepared by reducing quinone with sulphurous acid. It is a colourless crystalline solid melting at 169° and very easily soluble in water.

Quinone

Quinol is easily oxidised by ferric chloride to quinone, but quinone is usually prepared by oxidising aniline with potassium bichromate and sulphuric acid.

It is a yellow crystalline solid with a peculiar smell and melts at 116°. It is not very soluble in water, but dissolves in alcohol and ether. It is volatile in steam.

Quinone in some respects behaves as a diketone, but in other respects as an aromatic compound : it is generally represented by the

formula

in which there are two pairs of double bonds. On reduction, the centric formula is formed, but on oxidation it breaks down at the double linkings ; it combines with 2 or 4 atoms of bromine.
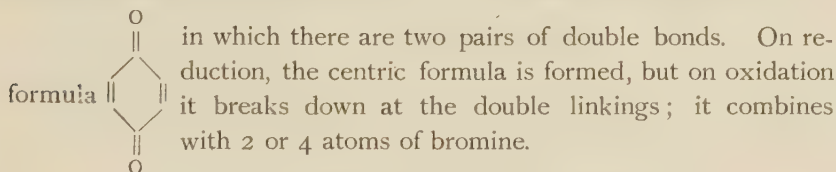
Reactions of Catechol, Resorcinol, Quinol.

* Aqueous solutions behave as follows :—

(1) With FeCl$_3$:—Catechol gives a green coloration ; this colour changes to violet, then to red on adding sodium carbonate, or ammonia. Resorcinol gives a deep violet coloration. Quinol, on boiling with ferric chloride, yields quinone with its peculiar irritating smell.

* (2) With bromine water :—Resorcinol gives a crystalline precipitate of tribromoresorcinol.

* (3) Catechol and quinol reduce ammoniacal silver nitrate.

* (4) Catechol and quinol reduce Fehling's solution.

* (5) Solutions of catechol and quinol, made alkaline with caustic soda, turn brown, firstly at the surface but, on shaking, throughout the whole solution. This is due to absorption of oxygen and oxidation.

* (6) With Millon's reagent :—Quinol gives a yellow colour and then a yellow precipitate which becomes red on heating.

Guaiacol and Veratrol.
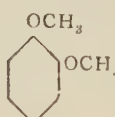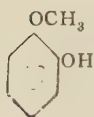
These compounds are methyl derivatives of catechol and are contained in natural substances. Guaiacol, like o-hydroxy-phenols, gives a coloration with ferric chloride; veratrol gives no coloration.

Phthalic Acids.

The three phthalic acids, or benzene dicarboxylic acids,
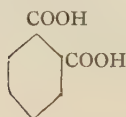
| Ortho, or phthalic acid, | Meta, or isophthalic acid, | Para, or terephthalic acid, |
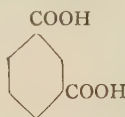
are obtained by oxidising the xylenes with nitric acid, or the toluic (methyl benzoic) acids with permanganate. Phthalic acid results from the oxidation of naphthalene.
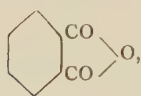
They are crystalline solids resembling benzoic acid. Phthalic acid, on heating, is converted into phthalic anhydride, a substance used in making fluorescein, eosin, and other dyes.

Nitrotoluenes, $C_6H_4 {\Large <} {}^{CH_3}_{NO_2}$.
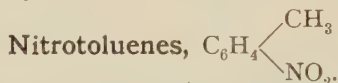
The o- and p-compounds are obtained by nitrating toluene; the m-compound is obtained by indirect methods. They are all solids.

Aminotoluenes, or Toluidines, $C_6H_4 {\Large <} {}^{CH_3}_{NH_2}$.
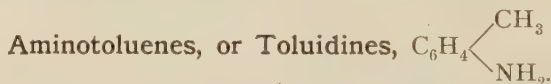
o- and p-Toluidine are obtained by reducing o- and p-nitrotoluene; o-toluidine is an oil, p-toluidine is a crystalline solid. The m-compound is obtained in a similar way and is an oil.

They yield diazonium salts with nitrous acid and behave generally like aniline.

Nitranilines, $C_6H_4 {\Large <} {}^{NO_2}_{NH_2}$.

m-Nitraniline is obtained by the partial reduction of m-dinitrobenzene with alcoholic ammonium sulphide. The o- and p-nitraniline cannot be obtained by nitrating aniline, but are obtained by nitrating acetanilide and saponifying the nitro-acetanilides.

Toluene-Sulphonic Acids, $C_6H_4 {\Large <} {}^{CH_3}_{SO_3H}$.

The o- and p-compounds result by sulphonating toluene, the o-compound being the chief product. They yield the cresols on fusion with potash.

Sulphanilic Acid, $C_6H_4{<}^{NH_2}_{SO_3H.}$

The p-compound is obtained by heating aniline sulphate at 200° for some hours.

It is a colourless crystalline solid easily soluble in hot water, very little in cold. It does not behave as a base, but the amino group can be diazotised. It is used largely in making dyes.

Cresols, $C_6H_4{<}^{CH_3}_{OH.}$

The three cresols are contained in the acid fraction of coal tar with phenol. Their separation is difficult to effect and they are prepared from the toluidines, or toluene sulphonic acids, by the methods given under aniline and diazonium salts and phenol.

p-Cresol occurs in urine in combination with sulphuric acid and is isolated, together with phenol, by the methods given on p. 185. It is also a product of the putrefaction of proteins and arises from the amino acid, tyrosine. The cresols are crystalline solids; o-cresol melts at 31°, m-cresol at 5°, p-cresol at 36°. They resemble phenol very closely in properties.

*   As phenols they react
(1) with ferric chloride;
(2) with bromine water;
(3) with nitric acid;
(4) with Millon's reagent.

Toluic Acids, $C_6H_4{<}^{CH_3}_{COOH.}$

These three acids result by oxidising the xylenes with dilute nitric acid. The o- and p-acids are most readily prepared from the toluidines by Sandmeyer's reaction with cuprous cyanide (p. 191).

They are solids resembling benzoic acid.

Anthranilic Acids, $C_6H_4{<}^{NH_2}_{COOH.}$
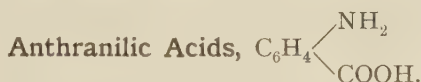
The o-acid was first obtained by oxidising indigo and is a colourless crystalline solid melting at 144°; it loses carbon dioxide on heating and yields aniline.

Sulpho-Benzoic Acids, $C_6H_4{<}^{SO_3H}_{COOH.}$

The o-acid is of interest as saccharin is prepared from it. o-Sulpho-benzoic acid is prepared by oxidising o-toluene-sulphonic acid. The ammonium salt, on heating, loses ammonia and gives the imide, saccharin :—

$$C_6H_4\begin{cases}CH_3\\SO_3H\end{cases} \rightarrow C_6H_4\begin{cases}COOH\\SO_3H\end{cases} \rightarrow C_6H_4\begin{cases}COONH_4\\SO_3NH_4\end{cases} \rightarrow C_6H_4\begin{cases}CO\\SO_2\end{cases}NH$$
Saccharin.

$$\rightarrow C_6H_4\begin{cases}CO\\SO_2\end{cases}N . Na$$
Sodium Salt.

Saccharin is a white crystalline solid melting at 224° and is only slightly soluble in water. It forms a sodium salt, which dissolves easily in cold water. The sodium salt, containing $2H_2O$ and crystallising in large plates, is generally used as sweetening agent. The sweetness of saccharin is about 500 times greater than that of cane sugar.

Saligenin, or Salicylic Alcohol, $C_6H_4\begin{cases}OH\\CH_2OH\end{cases}$.

Saligenin occurs as the glucoside, salicin, in the bark of the willow tree. The glucoside, on hydrolysis, gives glucose and saligenin.

It is generally prepared by reducing salicylic aldehyde with sodium amalgam and dilute alcohol.

Saligenin is a crystalline solid, which melts at 82° and is easily soluble in water. It is o-hydroxy-benzyl alcohol.

As it contains a phenolic group in the *a*-position, it gives a blue-violet colour with ferric chloride. It forms alkali salts with alkaline hydroxides and behaves like a phenol. It also behaves like a primary aliphatic alcohol and is converted on oxidation into salicylic aldehyde and salicylic acid.

Salicylic Aldehyde, $C_6H_4\begin{cases}OH\\CHO\end{cases}$.

o-Salicylic aldehyde is found in certain volatile oils from plants.

It can be prepared by oxidising saligenin with potassium bichromate and sulphuric acid.

It is generally prepared by Reimer's reaction: a mixture of phenol, chloroform, and caustic potash is heated under a reflux condenser :—

$$C_6H_5OH + CHCl_3 + 4KOH = C_6H_4(OK)CHO + 3KCl + 3H_2O.$$

The solution is acidified after distilling off the chloroform and distilled with steam; phenol and o-salicylic aldehyde pass over. The distillate is extracted with ether and the aldehyde converted into the bisulphite compound; this is decomposed with sodium carbonate, the salicylaldehyde extracted with ether and distilled.

p-Hydroxybenzaldehyde is also formed in the reaction, but is not volatile with steam.

Salicylic aldehyde is an oily liquid boiling at 196° with characteristic aromatic smell : it gives a violet colour with ferric chloride.

Salicylic Acids, $C_6H_4 \begin{cases} OH \\ COOH \end{cases}$

The chief of the hydroxy-benzoic acids is the o-compound, or salicylic acid, which occurs in the flowers of *Spiræa ulmaria* and in the form of its methyl ester in oil of winter green.

Salicylic acid was formerly prepared (1) by the hydrolysis of oil of winter green ; (2) by oxidising salicylic alcohol ; (3) by the action of nitrous acid on anthranilic acid.

It is now prepared almost entirely from phenol :—Sodium phenate is heated in carbon dioxide ; sodium phenyl carbonate is formed :—

$$C_6H_5ONa + CO_2 = C_6H_5 . O . COONa.$$
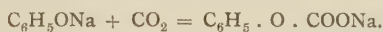
On heating, this is changed into sodium salicylate :—

$$2C_6H_5 . O . COONa = C_6H_5OH + C_6H_4 \begin{cases} ONa \\ COONa \end{cases} ;$$
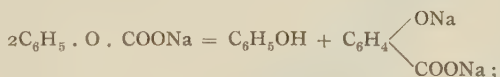
half the phenol used is recovered.

If the sodium phenyl carbonate be heated under pressure at 120-140°, it yields the acid salt of salicylic acid :—

$$C_6H_5 . O . COONa = C_6H_4 \begin{cases} OH \\ COONa \end{cases}.$$
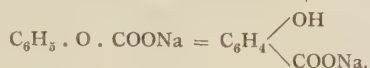
Salicylic acid forms colourless needles which melt at 155°. It is not easily soluble in cold water, but dissolves readily in hot water, alcohol, ether, and other organic solvents. It is an antiseptic, like phenol, and is used for preserving food-stuffs and also largely in medicine, more frequently in the form of its derivatives, aspirin and salol.

Salicylic acid behaves as an acid and as a phenol ; it dissolves in caustic alkali forming a salt with the carboxyl and phenolic groups ; in alkali carbonates forming a salt only with the carboxyl group.

Reactions.

(1) On heating, it melts and sublimes, but on further heating, it loses carbon dioxide and yields phenol.

The formation of phenol takes place more readily on heating with soda lime.

(2) As it is a phenol it gives the reactions :—

(*a*) With ferric chloride—a violet colour. This is discharged by mineral acids, but not by acetic acid. It may thus be distinguished from phenol.

Note.—Only the hydroxy acid containing the OH group in the
ortho position gives a violet colour with ferric chloride.   The
m- and p-compounds do not give colours with ferric chloride.

*   (*b*) With bromine water—a yellowish-white precipitate of dibromo-
and tribromosalicylic acids.

*   (*c*) With nitric acid—a yellow colour intensified on making the
solution alkaline with ammonia.

*   (*d*) With Millon's reagent—a red colour on heating.

Aspirin is acetyl-salicylic acid.   It is prepared by heating salicylic
acid with acetyl chloride, or acetic anhydride :—

$$C_6H_4\!\!\begin{array}{c} \diagup OH \\ \diagdown COOH \end{array} + CH_3COCl = HCl + C_6H_4\!\!\begin{array}{c} \diagup O.OC.CH_3 \\ \diagdown COOH. \end{array}$$
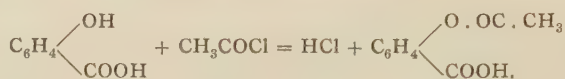
On hydrolysis by acids or alkalies, it yields acetic and salicylic acids.

Salol is phenyl salicylate.   It is prepared by heating a mixture of
sodium phenate and sodium salicylate with phosphorus oxychloride :—

$$2C_6H_4\!\!\begin{array}{c} \diagup OH \\ \diagdown COONa \end{array} + 2C_6H_5ONa + POCl_3 = 3NaCl + NaPO_3 + 2C_6H_4\!\!\begin{array}{c} \diagup OH \\ \diagdown COOC_6H_5. \end{array}$$
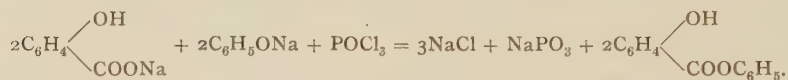
On hydrolysis, it yields phenol and salicylic acid.

If the hydrolysis of aspirin and salol be effected with caustic soda
the sodium salts are obtained; on acidifying with sulphuric acid, sali-
cylic acid is precipitated and the acetic acid, or phenol, can be isolated
by steam distillation.

$$\textbf{Tyrosine, } C_6H_4\!\!\begin{array}{c} \diagup OH \\ \diagdown CH_2.CH(NH_2).COOH. \end{array}$$
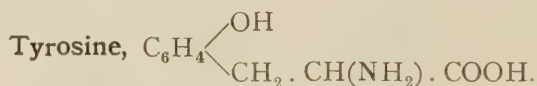
Tyrosine, or p-hydroxyphenylalanine, is a constituent of proteins
from which it was first obtained by Liebig in 1846 who fused cheese
τυρος) with caustic potash.   It has since been isolated from the pro-
ducts of hydrolysis of most proteins.   It is found in the liver and
other organs in certain diseases in considerable quantities; in minute
amounts it is present in all tissues.

Preparation.

The best yield of tyrosine is obtained from silk ; silk is hydrolysed by boiling
with concentrated hydrochloric acid for 5 or 6 hours, the solution is evaporated
to remove hydrochloric acid, the greater part of the remainder is removed as
cuprous chloride by adding cuprous oxide, and on neutralising the solution
tyrosine separates out.   It may also be obtained by hydrolysing silk and
other proteins with six times their amount of 30 per cent. sulphuric acid,
removing the sulphuric acid with baryta and concentrating the solution.
Tyrosine separates out and is recrystallised.

It is most easy to prepare tyrosine by the tryptic digestion of caseinogen. 100-500 gm. of caseinogen are dissolved in 2-10 litres of ·4 per cent. sodium carbonate, 1-2 gm. of dried pancreas (trypsin) are added and 1-2 per cent. of toluene, or chloroform, are shaken up with the solution to prevent putrefaction. The solution is kept at 35° for 7-10 days. It becomes cloudy with the separation of tyrosine which gradually settles out. The filtrate gives the reactions for tyrosine and, on evaporation and on cooling, deposits a further quantity. Almost pure tyrosine may be obtained from the first deposit by dissolving it in ·1N hydrochloric acid, boiling with charcoal to decolorise it, and exactly neutralising the clear solution with ammonia. Pure tyrosine may be obtained from the second deposit by the same treatment repeated two or three times.

Properties.

Tyrosine is a colourless crystalline solid, which is soluble with difficulty in cold water, more easily in hot. It dissolves readily in dilute acids, or alkalies, from which it separates on neutralising the solution.

If a small quantity of tyrosine be dissolved in a drop of ammonia on a glass slide and the ammonia be allowed to evaporate, the tyrosine crystallises out in characteristic bunches of fine needles (Fig. 29). It is insoluble in alcohol and ether.

Fig. 29.—Tyrosine.

Reactions and Tests.

(1) Its crystalline form is very characteristic.

(2) It gives a yellow colour on heating with nitric acid; this becomes orange on making alkaline with ammonia.

(3) It gives a red colour, even in extreme dilution, on heating with Millon's reagent.

(4) *Piria's test.*—3 drops of concentrated sulphuric acid are put on a little tyrosine in a dry test tube and it is placed in the boiling water-bath for half an hour. The red liquid is diluted with 10 c.c. of water and neutralised with barium carbonate. The filtrate (from $BaSO_4$) on evaporation to a small volume gives a violet colour with 2-3 drops of ferric chloride, showing the presence of phenol.

(5) When boiled with copper carbonate it gives a blue copper salt like aliphatic amino acids.

(6) *Mörner's test.*—A solution of tyrosine gives a green colour on boiling with a solution of formalin in sulphuric acid (1 vol. formalin, 45 vols. water, 55 vols. conc. H_2SO_4).

(7) A wine-red colour is formed if tyrosine be added to 3 or 4 drops of formalin in 5 c.c. of concentrated sulphuric acid. The liquid becomes green on adding double the volume of glacial acetic acid and boiling (*Denigès*).

Tyramine.

OH

CH₂
|
CH₂ . NH₂.
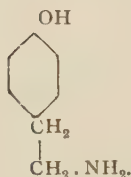
Tyramine, or p-hydroxyphenylethylamine, is a base formed from tyrosine by putrefaction. It occurs in ergot of which it is one of the active principles.

The decomposition of tyrosine by putrefaction is exactly similar to that of phenylalanine and takes place in the following stages :—

C_6H_4
OH
CH₂ . CH₂ . COOH
p-hydroxyphenyl propionic acid.

← C_6H_4
OH
CH₂ . CH(NH₂)COOH
Tyrosine.

→ C_6H_4
OH
CH₂ . CH₂ . NH₂
Tyramine.
→

C_6H_4
OH
CH₂ . COOH
p hydroxyphenyl acetic acid.

→

C_6H_4
OH
CH₃
p-cresol.

→

C_6H_5 . OH.

Phenol.
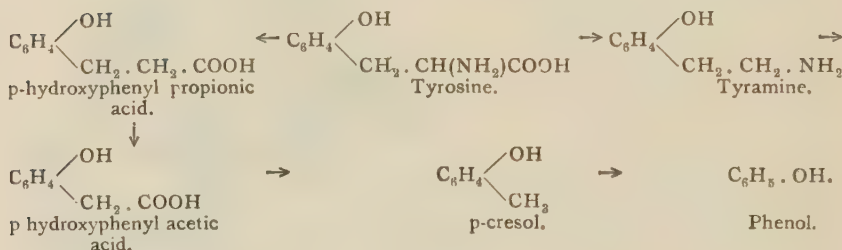
Thyroxine, $C_{15}H_{11}O_4NI_4$.

The active substance, thyroxine, of the thyroid gland, is a derivative of tyrosine. The fresh gland contains approximately 0·027 per cent. ; the dried gland about 0·12 per cent. Harington[1] has shown that thyroxine is an iodo derivative of the p-hydroxyphenyl ether of tyrosine :—

HO ⟨3 2 / 4 1 / 5 6⟩ —O— ⟨3' 2' / 4' 1' / 5' 6'⟩ CH₂ . CH(NH₂) . COOH.
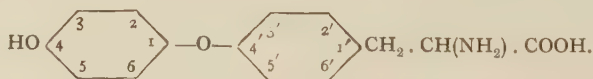
The position of the iodine atoms appears to be 3, 5, 3′, 5′.

[1] Biochem. J., 1926, **20**, 293, 300.

The two following other disubstitution products of benzene occur in nature :—

(1) **Cymene,** or methylisopropyl benzene, which occurs in numerous essential oils, can be obtained by heating camphor with phosphorus pentoxide, by heating turpentine with concentrated sulphuric acid and by reducing carvacrol and thymol with phosphorus pentasulphide.

CH_3

CH

CH_3 CH_3.

It is a colourless lipuid which boils at 175-176° and has a sp. gr. of ·8722 at 0°.   It yields p-toluic acid and terephthalic acid on oxidation.

(2) **Anethole,** or p-methoxyphenylpropylene, is the principal constituent of oil of aniseed.   It yields p-methoxybenzoic acid, or anisic acid, on oxidation with chromic acid, but anisic aldehyde, or p-methoxybenzaldehyde, on oxidation with bichromate and sulphuric acid.   Anisic aldehyde, on reduction with sodium amalgam and alcohol, is converted into anisic alcohol.

OCH_3

CH
$\parallel$
CH
$\mid$
CH_3

CHAPTER XXIV.

TRI- AND TETRA-SUBSTITUTION DERIVATIVES OF BENZENE.

TRISUBSTITUTION derivatives of benzene exist in the three forms :—

Vicinal.　　　　　Asymmetric.　　　　　Symmetric.
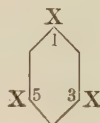
Four isomers are possible in the case of the tetrasubstitution derivatives.

The number of compounds in these groups is very large. Mention can only be made of those which occur naturally, or are prepared from natural sources.

Trihydric Phenols.

The trihydric phenols are :—
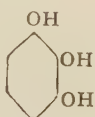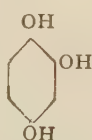
Pyrogallol.　　　　Hydroxyquinol.　　　Phloroglucinol.
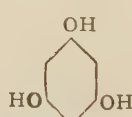
Pyrogallol, or pyrogallic acid, is obtained by heating gallic acid at 210° until carbon dioxide is no longer evolved :—

$$C_6H_2(OH)_3 . COOH = CO_2 + C_6H_3(OH)_3.$$
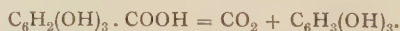
It is a colourless crystalline solid, which melts at 115°. It is easily soluble in water, but less in alcohol and ether. It will be noticed that the solubility of phenols in water increases with the number of hydroxyl groups.

Hydroxyquinol is obtained by fusing quinol with potash. It dissolves easily in water and melts at 140°.

Phloroglucinol results from the fusion of numerous resins with caustic potash. It is prepared by fusing resorcinol with caustic potash.

Phloroglucinol is a crystalline solid containing two molecules of water and melting at 218°. It is very soluble in water and is also soluble in alcohol and ether. In most respects it behaves as a trihydroxyphenol, but it forms an oxime and probably has also a ketonic structure.
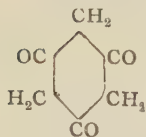
Reactions.

* (1) With ferric chloride, pyrogallol gives a deep-blue coloration; hydroxyquinol gives a greenish-brown colour which changes to blue, on adding sodium carbonate, and then to red; phloroglucinol gives a blue violet coloration.

* (2) In alkaline solution in contact with air, they absorb oxygen and the solution becomes brown. Pyrogallol is consequently used for absorbing oxygen.

* (3) They all reduce Fehling's solution and ammoniacal silver nitrate.

Orcinol is dihydroxy-toluene. **Thymol** and **carvacrol** are derivatives of cymene :—

Orcinol.   Thymol.   Carvacrol.
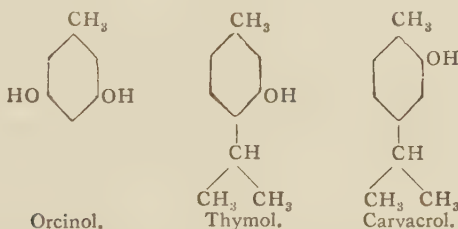
Protocatechuic acid, or catechol-carboxylic acid, results from the fusion of numerous resins, e.g. gum benzoin, catechin resin, with caustic potash. It is prepared by heating catechol with water and ammonium carbonate to 140°.
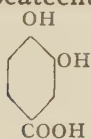
It is a colourless crystalline solid melting at 199° and dissolves readily in water.

With ferric chloride, its solution gives a green colour, which changes to blue and then to red on the addition of very dilute sodium carbonate. With ferrous sulphate, a violet colour is given. It is precipitated from solution by lead acetate.

Veratric acid, the dimethyl ether of protocatechuic acid, occurs in the seeds of *Veratrum sabadilla,* together with veratrine.

Vanillin is obtained from coniferyl alcohol by oxidation with chromic acid. Vanillin is the sweet-smelling constituent of the vanilla bean.

Coniferyl alcohol occurs in the glucoside, coniferin, and is obtained from it by hydrolysis.

OCH$_3$ / OCH$_3$ / COOH
Veratric acid.

OH / OCH$_3$ / CHO
Vanillin.
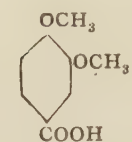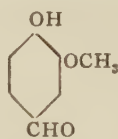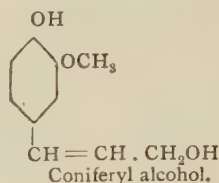
OH / OCH$_3$ / CH = CH . CH$_2$OH
Coniferyl alcohol.

Homogentisic Acid.

Homogentisic acid, or quinol-acetic acid, is found in the urine in the rare disorder known as alkaptonuria. Its presence is first shown by the urine turning brown and black on standing, or by its reducing power. It is a white crystalline solid melting at 146-147°.
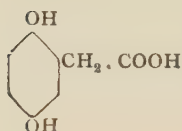
OH / CH$_2$. COOH / OH

As a derivative of hydroquinone it reacts with

(1) ferric chloride ;

(2) Fehling's solution, or ammoniacal silver nitrate ;

(3) turns black in the air in alkaline solution.

Adrenaline.

Adrenaline is a derivative of catechol and is the active principle of the adrenal gland from which it is prepared, as well as by synthesis.

The natural substance is laevorotatory : the synthetical is inactive ; the dextrorotatory form has only a very slight pharmacological action in comparison with the laevorotatory, or natural, form.

OH / OH / CHOH / CH$_2$. NH(CH$_3$).

Preparation.

Abel's method is probably the most convenient one for preparing adrenaline. To minced suprarenal glands in a series of flasks is added with thorough shaking an equal weight of 3·5 per cent. trichloracetic acid in alcohol. After 12 hours the mass is filtered. The filtrate is concentrated to about one-fiftieth and again filtered. Concentrated ammonia is added to the filtrate until the liquid just smells perceptibly of ammonia. Adrenaline is precipitated, filtered off, washed with water, alcohol, and ether. A yield of about ·2 per cent. is obtained. A further ·1 per cent. can be obtained by extracting the mass again with trichloracetic acid. It is recrystallised by solution in alcohol containing oxalic acid and precipitation by ammonia.

Properties.

Adrenaline is a colourless crystalline solid melting at 211-212°. It is not easily soluble in cold water, but more readily in hot water and is not soluble in most organic solvents.

It is a strong base and dissolves in mineral acids ; as a phenol it dissolves in caustic alkalies, but not in carbonates, or ammonia. Its aqueous solutions are not stable, but turn pink in the air.

Reactions.

(1) Ferric chloride in neutral, or faintly acid, solution gives a green coloration, which, on the careful addition of very dilute alkali, changes to violet, red-violet, and red.

(2) Oxidising agents and air produce a pink colour: potassium persulphate added up to ·1 per cent. to the solution of adrenaline gives a colour at a dilution of 1 in 5,000,000 (Ewins).

(3) Adrenaline gives an intense blue colour with Folin's phosphotungstic acid reagent for uric acid. One part in 3,000,000 parts of water gives a reaction with this reagent.

The estimation of adrenaline in the suprarenal gland is most easily effected by means of this colour reaction.

A full account of adrenaline is given in Barger's "Simpler Natural Bases."

Picric Acid.

Picric acid is trinitrophenol, a tetrasubstitution product of benzene.
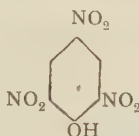
It is easily formed from phenol by the action of nitric acid (p. 186).

Gallic Acid.
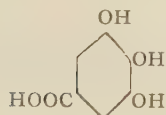
Gallic acid is present in gall nuts, tea, and other plants.

It is prepared by the hydrolysis of tannin.

It crystallises in silky needles which melt at 220°. It is not very soluble in cold water, but readily in hot water. It dissolves in alkalies and the solution turns brown in the air. It resembles pyrogallol in its reactions with ferric chloride, Fehling's solution, etc.

It does not precipitate gelatin and is not precipitated by lead acetate.

Tannic Acid, or Digallic Acid.
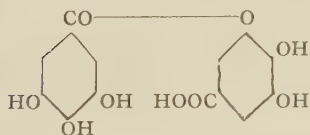
This acid, the anhydride of gallic acid, occurs in gall nuts, sumach, and other kinds of bark. It may be prepared by heating gallic acid with phosphorus oxychloride. Its constitution is not definitely known and it is sometimes referred to as tannin, but the synthetical product obtained from gallic acid has not the same properties as natural tannin which contains digallic acid in its constitution.

The natural product is here referred to as tannin, the synthetical as digallic acid.

Two other digallic acids are known.

IN addition to benzene and its substitution products, compounds in which only one benzene ring is present, numerous compounds are known which contain two or more benzene nuclei. These nuclei may be joined by means of one or more carbon atoms, or they may be joined together directly.

Diphenylmethane.
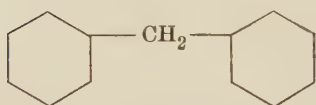
Diphenylmethane is the first and chief example of a complex aromatic compound in which two benzene rings are united by a carbon atom.

It is obtained by heating benzene with benzyl chloride in the presence of aluminium chloride :—

$$C_6H_5CH_2Cl + C_6H_6 = HCl + C_6H_5 . CH_2 . C_6H_5.$$

Diphenylmethane is a crystalline solid, which melts at $26 \cdot 5°$. It closely resembles benzene in forming nitro and other derivatives. On oxidation with chromic acid, it yields benzophenone, $C_6H_5 . CO . C_6H_5$.

Triphenylmethane.
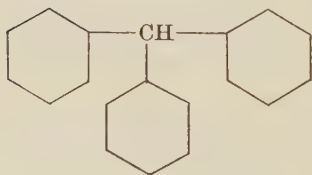
Triphenylmethane contains three benzene nuclei joined to one carbon atom, and is obtained by heating benzal chloride with benzene in the presence of aluminium chloride :—

$$C_6H_5 . CHCl_2 + 2C_6H_6 = 2HCl + C_6H_5 . CH \begin{cases} C_6H_5 \\ C_6H_5 \end{cases}$$
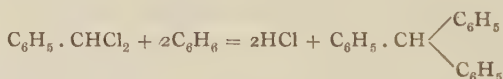
or by heating benzene with chloroform in the presence of aluminium chloride :—

$$3C_6H_6 + CHCl_3 = 3HCl + C_6H_5 . CH \begin{cases} C_6H_5 \\ C_6H_5. \end{cases}$$

Triphenylmethane is a colourless solid, which melts at $92°$ and boils at $358°$. It is not easily soluble in cold alcohol, but dissolves easily in ether and benzene.

Triphenylmethane is the parent substance of phenolphthalein and the group of rosaniline dyes (p. 223).

When oxidised with chromic acid it is converted into triphenylcarbinol $(C_6H_5)_3 . C . OH$.

Diphenyl.
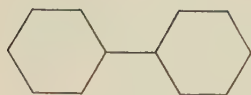
Diphenyl is the first instance of aromatic compounds in which the benzene rings are directly joined together by a single bond. It is prepared by treating an ethereal solution of bromobenzene with sodium :—

$$C_6H_5Br + Na_2 + C_6H_5Br = 2NaBr + C_6H_5—C_6H_5,$$
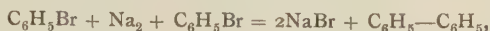
or it is formed when benzene vapour is passed through a red-hot tube.

It is a colourless solid melting at 71° and boiling at 254°; it closely resembles benzene in its reactions. On oxidation, it yields benzoic acid.

Azoxybenzene.
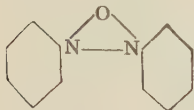
When nitrobenzene is reduced with alkaline reducing agents, such as sodium and alcohol, it yields azoxybenzene. Azoxybenzene is a yellow crystalline solid melting at 36°. It is insoluble in water, but soluble in alcohol, ether, and organic solvents.

Azobenzene.
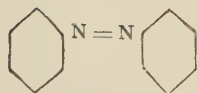
Azobenzene is formed when azoxybenzene is carefully distilled with three parts of iron filings.

It is a brilliant red crystalline solid, which melts at 68° and distils at 293°. It is not soluble in water, but dissolves in organic solvents.

Hydrazobenzene.

When azobenzene is reduced with alkaline reagents, ammonium sulphide, or zinc and caustic soda, it is converted into hydrazobenzene.

Hydrazobenzene forms colourless crystals, which melt at 131°. It is reduced by zinc dust and acetic acid to aniline.

Benzidine.

Benzidine, or diamino-diphenyl, is formed when hydrazobenzene is treated with concentrated hydrochloric acid; intramolecular rearrangement takes place.

It may be obtained by treating azobenzene with tin and concentrated hydrochloric acid.

Benzidine forms colourless crystals which melt at 128°.

It is a base like aniline, forming salts with acids. The sulphate is very insoluble and is used for estimating sulphates.

It is diazotised by nitrous acid and, like aniline, is used largely in the preparation of dyes.

NAPHTHALENE.

Naphthalene, the hydrocarbon which is present in coal tar in the largest quantity, is present in the second fraction, boiling from 170-230°, when the tar is fractionally distilled.

This fraction on cooling deposits crystals of naphthalene. These are separated from phenols, etc., by pressure. The impure mass is shaken with caustic soda to remove the remainder of the phenols, washed and warmed with sulphuric acid, which sulphonates the impurities, dissolving them. The naphthalene is obtained by distillation, or sublimation.

Naphthalene is a colourless solid crystallising in shining plates which melt at 79° and boil at 218°. It has a characteristic smell and is extremely volatile. All the naphthalene formed by the distillation of coal is not condensed and a portion reaches the gas mains and gas pipes; in cold weather the naphthalene crystallises out and may cause blocking of the pipes. It does not dissolve in water, but it dissolves easily in hot alcohol, ether, and other organic solvents. It combines with picric acid, like other complex hydrocarbons, to form a yellow crystalline solid which melts at 149.°

When naphthalene is oxidised by dilute nitric acid, or chromic acid, it yields o-phthalic acid. On nitration, it yields α-nitronaphthalene; α-amino-naphthalene is formed by the reduction of the nitro compound. Nitronaphthalene, on oxidation, gives nitrophthalic acid: aminonaphthalene gives phthalic acid. The formation of phthalic acid shows the presence of one benzene ring with substituting groups in the o-position. The same is shown by the oxidation of nitronaphthalene, but the oxidation of aminonaphthalene shows that a different benzene ring is oxidised to that which is oxidised in the case of nitronaphthalene. Naphthalene would thus contain two benzene rings joined together in the ortho-position as represented by the formula :—
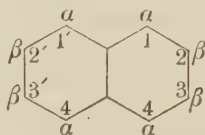
This constitution is proved by synthesis and emphasised by the similarity in behaviour of naphthalene to benzene.

Derivatives of Naphthalene.

Naphthalene resembles benzene in forming nitro, sulphonic acid, amino-derivatives. Two monosubstitution derivatives can be formed, the α and the β forms. In some cases both are formed, in other cases only one is formed. The accompanying formulæ show how these derivatives are prepared from naphthalene :—
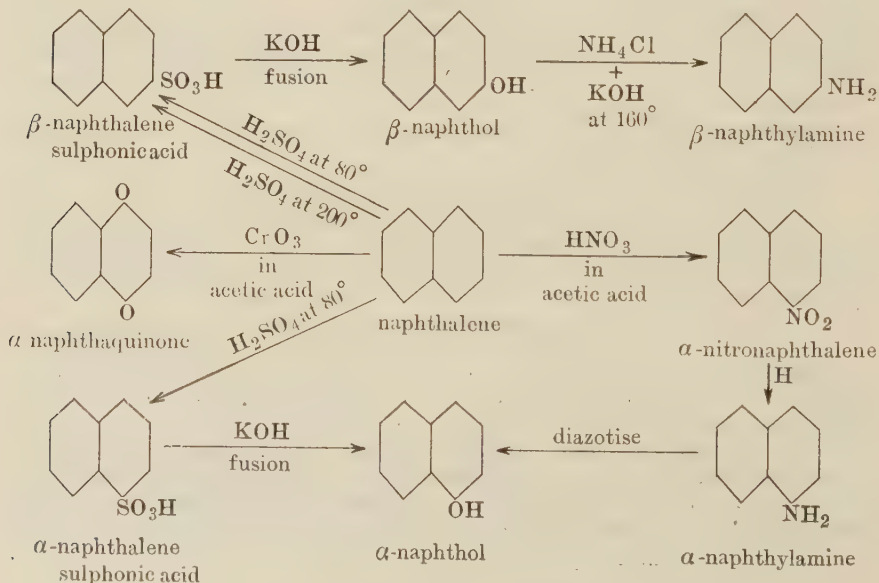
The naphthalene sulphonic acids are crystalline hygroscopic solids.

α-naphthol is crystalline, melts at 95°, and boils at 280°; it resembles phenol in smell, is only slightly soluble in water, but dissolves easily in alcohol and ether. It gives a violet flocculent precipitate with ferric chloride.

β-naphthol is a crystalline solid, melting at 138°. It forms nitro derivatives like phenol, many of which are used as dyes.

α-naphthylamine is a colourless, crystalline solid melting at 50° and boiling at 300°. It has an unpleasant smell and turns red on exposure to air; on oxidation it is converted into *α*-naphthaquinone. It gives a blue precipitate with ferric chloride.

β-naphthylamine is a colourless crystalline solid, melting at 112° and boiling at 294°. It has no smell and gives no precipitate with ferric chloride. It gives phthalic acid on oxidation.

α-nitronaphthalene is yellow, melts at 61° and boils at 304°.

β-nitronaphthalene, prepared by indirect methods, melts at 79°.

The chief derivatives of naphthalene are the naphthol sulphonic acids and naphthylamine sulphonic acids of which 14 isomers of each are possible.

Naphthalene and its substitution products are extensively used in the preparation of dyes. Apparently no natural compound contains a naphthalene ring.

Anthracene.
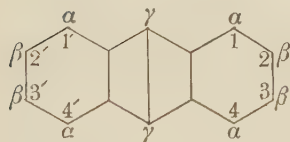
The chief interest attaching to anthracene is that it is the parent hydrocarbon from which the red dye of madder root is derived.

Anthracene is a constituent of coal tar and is isolated like naphthalene from the fraction boiling above 270.°

Its constitution has been arrived at by its resemblance in properties to benzene and naphthalene and by its synthesis, by treating tetrabromo-ethane with benzene in presence of aluminium chloride :—

$$C_6H_6 + \begin{matrix} BrCHBr \\ | \\ BrCHBr \end{matrix} + C_6H_6 = 4HBr + C_6H_4 \begin{matrix} CH \\ | \\ CH \end{matrix} C_6H_4.$$
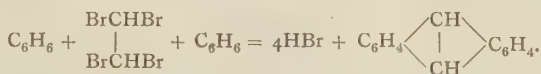
It is a colourless crystalline solid with a blue fluorescence, melting at 213° and boiling at 351°. It is insoluble in water, very slightly soluble in alcohol and ether, but easily soluble in benzene. .

Anthracene resembles benzene and naphthalene fairly closely, but it is oxidised by nitric acid and converted into anthraquinone.

Anthraquinone.
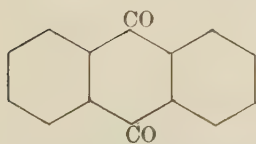
Anthraquinone is prepared by oxidising anthracene with sodium bichromate and sulphuric acid and is the chief derivative of anthracene.

It consists of pale yellow needles which melt at 285°. It resembles the aromatic ketones, e.g. benzophenone, rather than quinone and is a very stable compound. It is used in making alizarin and other dyes.

Alizarin.
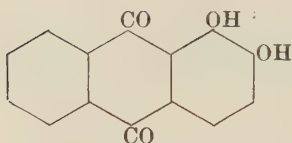
Alizarin occurs in madder root as the glucoside termed ruberythric acid. This glucoside on hydrolysis by enzymes, or by acids, gives two molecules of glucose and alizarin, the "Turkey-red" dye. Since its synthesis by Graebe and Liebermann, who determined its constitution, alizarin is made entirely from anthraquinone.

Anthraquinone is converted by sulphonation with sulphuric acid at 250°, or by heating with fuming sulphuric acid at 160°, into anthraquinone-β-monosulphonic acid. The solution is diluted with water, unchanged anthraquinone is filtered off and the solution neutralised with soda. Sodium anthraquinone-β-monosulphonate crystallises out and is purified by crystallisation. It is heated with caustic soda and a small quantity of potassium chlorate; the sodium salt of alizarin is obtained from which alizarin is precipitated on acidifying :—

$$C_6H_4 \Big\langle {CO \atop CO} \Big\rangle C_6H_4 + H_2SO_4 = C_6H_4 \Big\langle {CO \atop CO} \Big\rangle C_6H_3 . SO_3H + H_2O$$

$$C_6H_4 \Big\langle {CO \atop CO} \Big\rangle C_6H_3 \ SO_3Na + 3NaOH + O = C_6H_4 \Big\langle {CO \atop CO} \Big\rangle C_6H_2(ONa)_2 + Na_2SO_3 + 2H_2O.$$
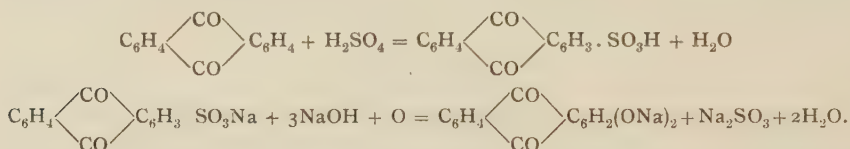
Alizarin forms dark red prisms melting at 290°. It is almost insoluble in water, slightly soluble in alcohol.

As a phenol, it forms salts with alkalies; the solution in caustic soda has a violet colour; the salts with the divalent and trivalent metals are insoluble and of various colours; these salts are the dyes which colour the fabrics.

Purpurin.
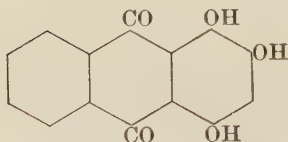
Purpurin, or 1, 2, 4-trihydroxy-anthraquinone is present in madder root with alizarin and is made by oxidising alizarin with manganese dioxide and sulphuric acid. It forms dark red needles, which melt at 253°; in dyeing, it gives yellower shades than alizarin.

Anthropurpurin   and   Flavopurpurin
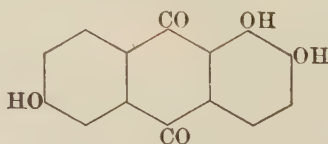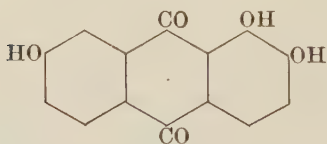
are two other anthraquinone derivatives used as dyes. It may be noted that only those derivatives containing two hydroxy groups in the 1, 2 position form dyes.

Mention may finally be made of the following complex aromatic compounds :—
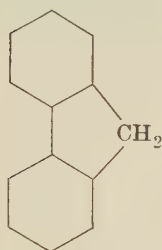
Fluorene.   Phenanthrene.   Chrysene.   Picene.
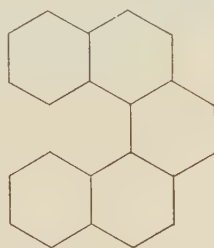
DYES.

Most of the dyes in common use are derivatives of the complex aromatic compounds. They may be divided into the following groups :—

(1) Nitro compounds.
(2) Triphenylmethane compounds :
   (a) basic.
   (b) acidic.

(3) Azo compounds.
(4) Phenols.
(5) Indigo (p. 351).

Nitro Compounds.

The nitro compounds are yellow dyes, mainly used for dyeing silk and wool, and on this account, since cotton is the chief material which requires dyeing, are few in number.

Picric acid dyes silk and wool, but not cotton, as can be easily verified by dipping silk, or wool, and cotton into picric acid solution, removing and washing. The silk, or wool, is dyed, the cotton is not dyed.

Martius yellow, dinitro-α-naphthol, is the chief nitro compound used for dyeing silk and wool. The commercial substance is the sodium salt.

Naphthol yellow is the sulphonic acid of Martius yellow. The potassium salt is the commercial dye.

Triphenylmethane Compounds.

Malachite green is prepared by heating a mixture of benzaldehyde and dimethylaniline with zinc chloride :—

$$C_6H_5 . CHO + 2C_6H_5 . N(CH_3)_2 = C_6H_5 . CH\begin{subarray}{l} C_6H_5N(CH_3)_2 \\ \\ C_6H_5N(CH_3)_2 \end{subarray} + 2H_2O.$$
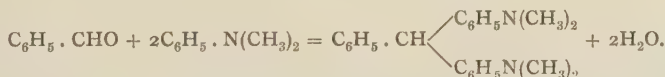
It dyes silk and wool a bluish-green; but cotton only after mordanting (p. 226).

Brilliant green is prepared in the same way using diethylaniline :—

$$C_6H_5 . CHO + 2C_6H_5N(C_2H_5)_2 = C_6H_5 . CH\begin{subarray}{l} C_6H_5N(C_2H_5)_2 \\ \\ C_6H_5N(C_2H_5)_2 \end{subarray} + 2H_2O.$$
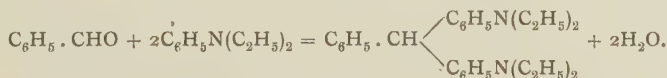
Acid green is prepared from benzaldehyde and ethylbenzylaniline :—

$$C_6H_5CHO + C_6H_5N(C_2H_5)C_7H_7 = C_6H_5 . CH\begin{subarray}{l} C_6H_5N(C_2H_5)C_7H_7 \\ \\ C_6H_5N(C_2H_5)C_7H_7 \end{subarray} + 2H_2O.$$
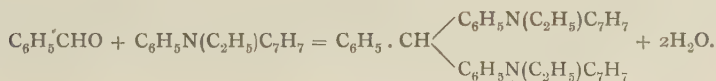
It dyes silk and wool in an acid solution, but is not used for dyeing cotton.

Pararosaniline is prepared by oxidising a mixture of p-toluidine and aniline with arsenic acid, or nitrobenzene. Probably, the p-toluidine is first oxidised to the aldehyde :—

$$H_2N . C_6H_4 . CHO + 2C_6H_5NH_2 = H_2N . C_6H_4 . CH{\Large\langle}^{C_6H_4 . NH_2}_{C_6H_4 . NH_2} + 2H_2O.$$
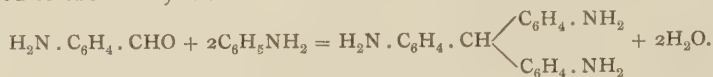
On further oxidation, it gives the carbinol :—

$$H_2N . C_6H_4 . \underset{\underset{OH}{|}}{C}{\Large\langle}^{C_6H_4 . NH_2}_{C_6H_4 . NH_2}.$$
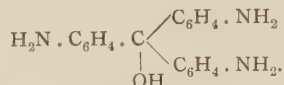
Rosaniline, Fuchsine or Magenta.

This compound is prepared by oxidising a mixture of p-toluidine, o-toluidine and aniline as above :—

$$H_2N . C_6H_4 . CHO + \left\{{C_6H_5NH_2 \atop H_2N . C_6H_4 . CH_3}\right\} = H_2N . C_6H_4 . CH{\Large\langle}^{C_6H_4NH_2}_{C_6H_3(CH_3)NH_2} + 2H_2O$$

$$\downarrow$$

$$H_2N . C_6H_4 . \underset{\underset{OH}{|}}{C}{\Large\langle}^{C_6H_4NH_2}_{C_6H_3(CH_3)NH_2}$$
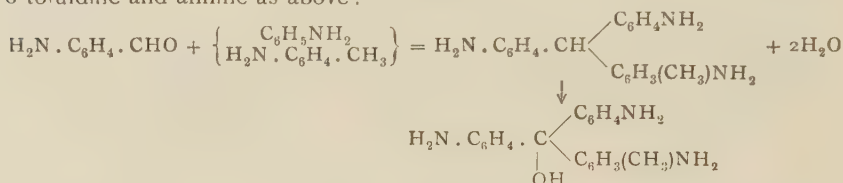
Pararosaniline and rosaniline are reddish-blue dyes.

These compounds on heating with methyl iodide become methylated. The colour becomes bluer. It is still more blue when ethyl iodide is used, and pure blue when phenyl groups are introduced, e.g. *aniline blue.*

$$C_6H_5 . NH . C_6H_4 . \underset{\underset{OH}{|}}{C}{\Large\langle}^{C_6H_4 . NH . C_6H_5}_{C_6H_3(CH_3)NH . C_6H_5}.$$
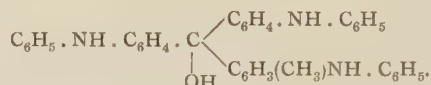
Phenolphthalein is prepared by condensing together phenol and phthalic anhydride :—

$$C_6H_4{\Large\langle}^{CO}_{CO}{\Large\rangle}O + 2C_6H_5OH = C_6H_4{\Large\langle}^{C-C_6H_4OH}_{CO}{\Large\rangle}O$$

where the upper group carries $\overset{C_6H_4OH}{|}$
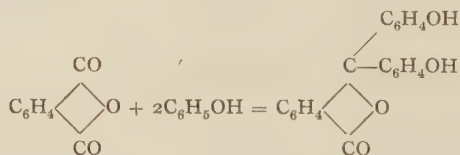
Fluorescein is obtained by condensing resorcinol with phthalic anhydride.

Eosin is obtained by condensing dibromoresorcinol with phthalic anhydride. It is a tetrabromofluorescein.

Erythrosin is obtained by condensing di-iodoresorcinol with phthalic anhydride. It is tetra-iodofluorescein.

These dyes have a magnificent greenish fluorescence and are mostly used for dyeing silk. Eosin is used as red ink, phenolphthalein is the well-known indicator. The phenol acid is colourless, the alkaline salt is coloured.

Constitution of Triphenylmethane Dyes. The Theory of Colour.

An examination of the coloured aromatic compounds has shown that they contain particular groupings, e.g. the nitro-group, the azo-group.

Quinones are also coloured compounds. The triphenylmethane derivatives are neither nitro-compounds nor azo-compounds; they are believed to have a quinonoid structure :—

$C_6H_5 \cdot C(OH) \cdot C_6H_4 \cdot N(CH_3)_2$

$N:(CH_3)_2$
H   Cl

Colourless leuco base.

$C_6H_5 \cdot C \cdot C_6H_4 \cdot N(CH_3)_2$

$Cl-N:(CH_3)_2$

Coloured base.

$HO \cdot C_6H_4 \cdot C \cdot C_6H_4 \cdot CO$

O

OH
Colourless anhydride
of
phenolphthalein.

$HO \cdot C_6H_4 \cdot C \cdot C_6H_4 \cdot COONa$

O

Coloured sodium salt.
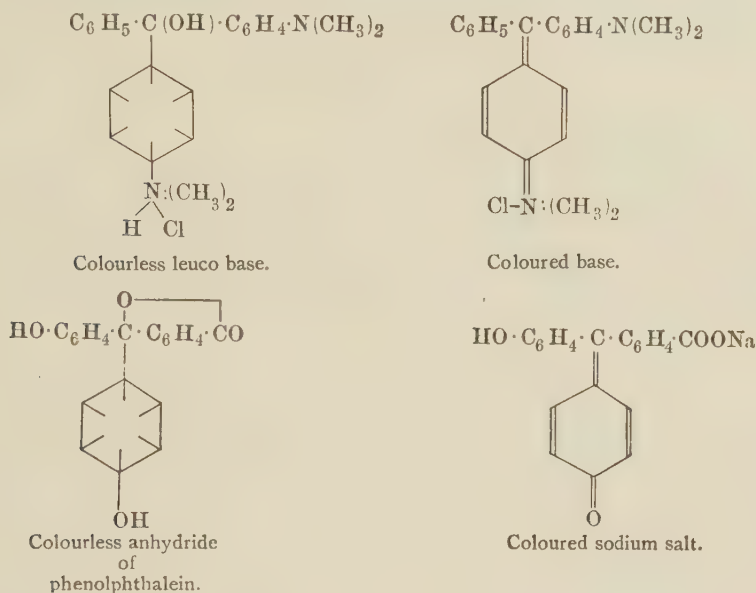
Malachite green, as prepared above, is a colourless crystalline solid and is known as the leuco base; on oxidation, it is converted into the green compound :—

$$C_6H_4 \cdot CH \begin{cases} C_6H_4 \cdot N(CH_3)_2 \\ C_6H_4 \cdot N(CH_3)_2 \end{cases} + O = C_6H_4 \cdot C \cdot OH \begin{cases} C_6H_4 \cdot N(CH_3)_2 \\ C_6H_4 \cdot N(CH_3)_2 \end{cases}$$
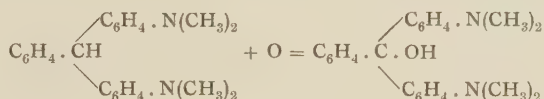
which is known as the colour base.

The colour bases of the rosanilines are formed directly ; on reduction, they give the leuco base.

These colour bases form salts with acids, e.g.

$$C_{23}H_{26}N_2O + HCl = C_{23}H_{25}N_2Cl + H_2O.$$
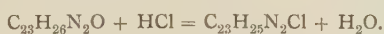
Azo Dyes.

Diazonium salts are prepared by treating aniline and other aromatic amines with nitrous acid. Besides yielding phenol on boiling and giving various derivatives by the Sandmeyer reaction, these diazonium salts have the property of combining with amines and phenols :—

$$C_6H_5 \cdot N:N \cdot OH + C_6H_5 \cdot NH_2 = C_6H_5 \cdot N:N-C_6H_4 \cdot NH_2 + H_2O$$
$$C_6H_5 \cdot N:N \cdot OH + C_6H_5 \cdot OH = C_6H_5 \cdot N:N-C_6H_4 \cdot OH + H_2O.$$
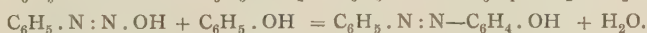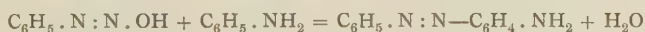
By means of this reaction an enormous number of dyes can be prepared which are either basic (aniline component), or acidic (phenol component). The following are examples :—

Helianthin from diazotised sulphanilic acid and dimethylaniline.
Chrysoidin from diazotised aniline and m-phenylenediamine.

Bismarck brown from diatotised m-phenylenediamine and m-phenylenediamine.

Resorcin yellow from diazotised sulphanilic acid and resorcinol.

Congo red from diazotised benzidine and naphthionic acid (naphthylamine sulphonic acid).

The free acid is blue in colour, the salts are red.

The Process of Dyeing.

There are many coloured substances amongst the aromatic compounds which are not dyes, e.g. dinitrobenzene is yellow, azobenzene is red, but the chief essential that a coloured substance should be a dye is that it should form an insoluble compound, which cannot be washed out by water, upon the fabric, or material, to be dyed. It will be noticed that the dyes above mentioned are either basic, or acidic, substances and are thus capable of forming salts with alkalies, or with acids.

Silk and wool are proteins and are compounds of the nature of amino acids, i.e. they are both basic and acidic in their properties. Most dyes combine with them and give insoluble salts. Cotton is a carbohydrate and forms no compounds with acids and bases. Cotton can, however, be dyed by mordanting, i.e. impregnating the fabric with an acid such as tannic acid, or a base such as alumina, ferric oxide, etc. Basic dyes form insoluble tannates, acidic dyes form insoluble salts, or lakes. In calico printing, the pattern is marked out with the mordant in a thick solution, such as gum, to prevent it from spreading.

The formation of insoluble salts upon the fabric is the chief method of producing insoluble deposits upon the fibre, but most probably the following method occurs at the same time. The dyes are all compounds of high molecular weight and form colloidal solutions (suspensions of the finest particles). The fabric, whether it consists of silk, wool, or cotton, is a colloid. The process of precipitation of colloids from solution by means of electrolytes, especially those with trivalent ions, and the process of mutual precipitation of two colloids will also be concerned in the fixing of the dye upon the fabric.

The Anthoxanthins.

The yellow plant pigments usually called flavones and xanthones are grouped together as the anthoxanthins, the term used in 1835 by Marquart and again suggested by Willstätter in 1913 on account of their similarity to the anthocyanins.

The anthoxanthins contain the complex benzene-γ-pyrone nucleus.

The γ-pyrone nucleus is the heterocyclic six-membered ring containing an oxygen atom and a keto group in the para- or γ-position to the oxygen atom. The keto group in this ring does not behave in all respects like a ketone group; it forms no derivative with hydroxylamine. The oxygen atom is also peculiar in its properties; it is basic and combines with mineral acids to form salts, the oxygen atom becoming quadrivalent.
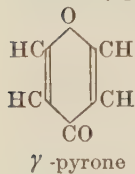
Flavone and xanthone are phenyl derivatives of benzo-γ-pyrone. The yellow pigments are hydroxy derivatives of these compounds and of flavonol.
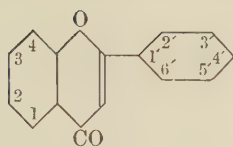
Flavone.
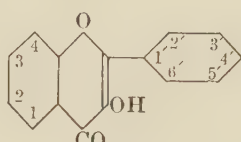
Flavonol,
or
hydroxyflavone.
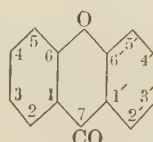
Xanthone.

The constitution of some of the anthoxanthins has been shown to be the following :—
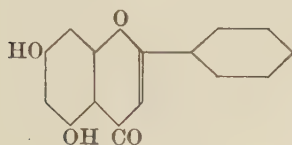
Chrysin,
or
1, 3-dihydroxyflavone.
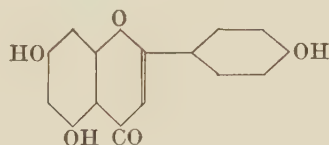
Apigenin,
or
1, 3, 4'-trihydroxy-
flavone.
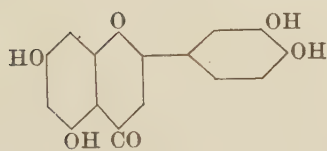
Luteolin,
or
1, 3, 3', 4',
tetrahydroxy
flavone.
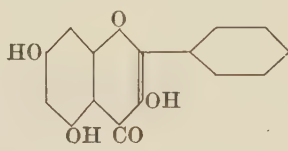
Galangin,
or
1, 3-dihydroxyflavonol.
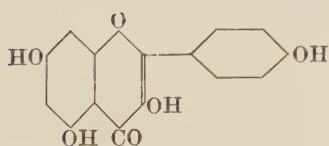
Caempferol,
or
1, 3, 4'-trihydroxy-
flavonol.
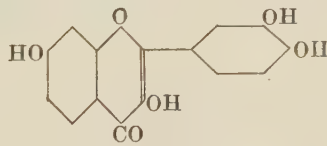
Fisetin,
or
3, 3', 4'-trihydroxy-
flavonol.
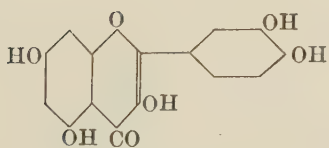
Quercitin,
or
1, 3, 3' 4'-tetrahydroxy-
flavonol.
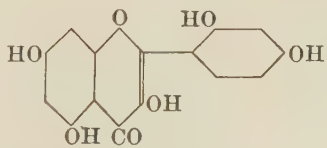
Morin,
or
1, 3, 2' 4'-
tetrahydroxy-
flavonol.

15 *
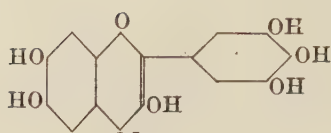
Myricetin,
or
2, 3, 3', 4', 5'-
pentahydroxy-flavonol.
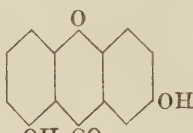
Euxanthone,
or
2, 3'-dihydroxy-
xanthone.
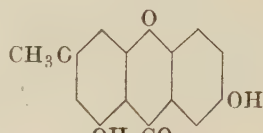
Gentisin,
or
4-methoxy-
2, 3'-dihydroxy-
xanthone.

These pigments thus contain the simple aromatic compounds, benzene, phenol, catechol, resorcinol, or pyrogallol, combined with benzo-γ-pyrone. On decomposition, they yield polyhydroxybenzoic acids (protocatechuic, hydroxybenzoic, or resorcylic) and the above phenols. Their formulæ have been arrived at by the study of these decomposition products and have been proved by synthesis. The details of the products of decomposition and the synthesis are given in the larger text-books of organic chemistry.

These pigments are yellow crystalline solids, very slightly soluble in water. They dissolve in acids giving yellow, or yellowish-red, solutions; they also dissolve in alkalies giving solutions which are yellow, or reddish. Some of them give a dull green, or red-brown, coloration with ferric chloride.

The Anthocyanins.

The red, violet, and blue pigments which are present in the blossoms, in many fruits and in some leaves of plants, and which can be extracted with water, or aqueous alcohol, are grouped together as the Anthocyanins. According to Molisch they occur not only dissolved in the juices of the cell, but also in amorphous and crystalline forms. Molisch obtained crystals by allowing aqueous, or acetic acid, extracts to evaporate slowly under the cover slip of a microscope slide. The colour of the aqueous solution of these pigments fades on standing, from which it would appear that the pigments are very unstable.
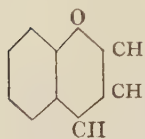
The chemical investigation of these pigments has shown that they are glucosides and are converted by hydrolysis with acids into glucose and anthocyanidins. The anthocyanidins are related to the group of anthoxanthins. These facts were first elucidated by Willstätter and his pupils in 1913. The anthocyanidins are derivatives of the complex benzo-pyrylium nucleus. This nucleus differs from the benzo-γ-pyrone nucleus in having a CH group in the place of the CO group.
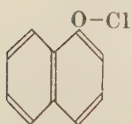
The properties of this group differ markedly from those of the benzopyrone group. The oxygen atom in the pyrone ring can become quadrivalent and form salts with acids; it is feebly basic; the salts are unstable and are decomposed by water. The oxygen atom in the benzopyrylium ring can also be quadrivalent and form salts with acids. It is strongly basic; the salts are stable and not easily decomposed by water.

The benzene ring contains hydroxyl groups; their phenolic character allows of the formation of salts with alkali. These properties explain the existence of the red, violet, and blue colours; the red is the acid salt, the blue is the potassium, or metallic, salt, and the violet is the anhydride of the pigment; thus the colour is due to the quinonoid structure of the molecule. The
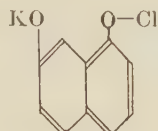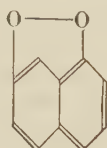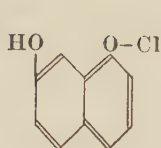
apparent instability of the compounds from the disappearance of the colour of the solutions is not confirmed. The colour returns on acidifying, or on evaporating the solution. The pigment apparently undergoes an isomerisation.

The anthocyanidins are pigments like the anthocyanins, but they are soluble in amyl alcohol.

The following anthocyanins have been investigated :—

(1) Cornflower and rose—
cyanin $\rightarrow$ 2 × glucose + cyanidin.
(2) Cranberry—
idain $\rightarrow$ galactose + cyanidin.
(3) Blue grapes—
oenin $\rightarrow$ glucose + oenidin.
(4) Whortle berry and *Althæa rosea*—
myrtillin $\rightarrow$ glucose + myrtillidin.
(5) Larkspur—
delphinin $\rightarrow$ 2 mol. glucose + 2 mol. p-oxybenzoic acid + delphinidin.
(6) Pelargonium—
pelargonin $\rightarrow$ 2 × glucose + pelargonidin.

The anthocyanidins are closely related to the anthoxanthins :—

Cyanidin is isomeric with luteolin, caempferol and fisetin.

Pelargonidin is isomeric with apigenin and galangin.

Delphinidin is isomeric with quercetin and morin.

Cyanidin, on heating with alkali, gives phloroglucinol and protocatechuic acid.

Pelargonidin, on heating with alkali, gives phloroglucinol and p-hydroxy-benzoic acid.

Delphinidin, on heating with alkali, gives phloroglucinol and gallic acid.

The constitution of these anthocyanidins is probably :—

Pelargonidin.

Cyanidin.

Delphinidin.

Oenidin.
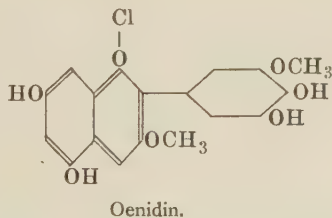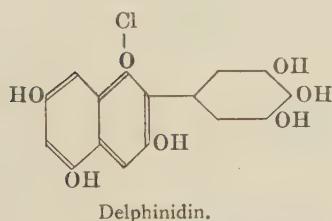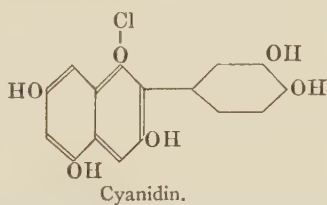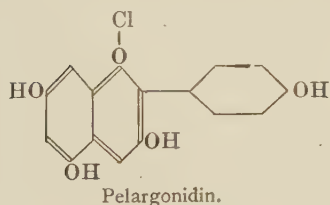
Though these compounds are so closely related to the anthoxanthins, the transformation of the one group into the other is not easily effected, but

quercitin on reduction has been converted into cyanidin. It is very likely that the two groups of compounds are converted into one another by oxidative and reducing enzymes.

Tannins.

The various kinds of tannin which can be extracted from gall nuts, sumach, pomegranate, oak bark, kino, etc., seem to belong to two main groups according to the reactions which they give with ferric chloride, bromine water, etc. They appear to contain a pyrogallol nucleus, or a catechol nucleus, thus :—

	Pyrogallol variety	Catechol variety
Ferric salts . .	Dark blue	Greenish-black (ink).
Bromine water .	No precipitate	Yellow, or brown, precipitate.
Leather .	Produce a "bloom"	No bloom.
Conc. H_2SO_4 .	—	Dark red ring at junction of liquids.

Some of the tannins of the catechol variety, gambier and cutch, appear to contain a phloroglucinol nucleus, since a pine shaving moistened with an extract and treated with concentrated hydrochloric acid gives a red, or purple, stain which is characteristic of phloroglucinol.

Constitution.

The constitution of the various tannins has not yet been definitely determined, but the work of Emil Fischer and his pupils indicates that the tannins are esters of glucose with gallic acid, digallic acid and other complex phenolic acids.

Fischer and his pupils have definitely shown that the tannin from Aleppo, Chinese, and other galls contains 7-8 per cent. of glucose. The yield of glucose corresponds very closely to that which would be obtained, if tannin were the pentadigalloyl ester of glucose. This constitution, though not absolutely proved, has been made extremely probable by synthesis. Fischer has prepared esters of glucose with a series of these phenolic acids and they possess the main properties of the tannins.

Tannins would therefore be constituted thus :—

$$
\begin{array}{l}
CH_2O\text{———}t \\
| \\
CHO\text{———}t \\
| \\
CH \\
| \\
CHO\text{———}t \\
| \\
CHO\text{———}t \\
| \\
CHO\text{———}t
\end{array}
$$
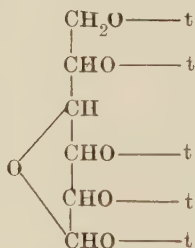
where t represents a complex acid like tannic acid, or digallic acid.

These compounds correspond to the fats in which glycerol is the basis and various fatty acids are combined with it.

This constitution not only allows for a large number of varieties of tannins where t is the same throughout the molecule, but also where t is different.[1]

In addition to the interest attached to the syntheses of a tannin there is another interest. Some of the synthetical tannins possess very high molecular weights; one of them has a higher molecular weight, 2051, than any other known synthetical compound, and has largely exceeded the figure of 1213, which is the molecular weight of the synthetical octadecapeptide (p. 368).

[1] A summary of the researches on tannins is given in the Ber. deutsch. Chem. Ges., 1913, **46**, 3253.

CHAPTER XXVI.

THE POLYHYDRIC ALCOHOLS.

THE polyhydric alcohols are a series of compounds containing in their molecules several hydroxyl groups attached to different carbon atoms. According to the number of hydroxyl groups, they are called di-, tri-, tetra-, poly-hydric alcohols. The chief representatives are the compounds containing hydroxyl groups on each carbon atom. Thus :—

$$
\begin{array}{lllll}
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
| & | & | & | & | \\
\text{CH}_2\text{OH} & \text{CHOH} & \text{CHOH} & \text{CHOH} & \text{CHOH} \\
\text{Glycol.} & | & | & | & | \\
 & \text{CH}_2\text{OH} & \text{CHOH} & \text{CHOH} & \text{CHOH etc.} \\
 & \text{Glycerol.} & | & | & | \\
 & & \text{CH}_2\text{OH} & \text{CHOH} & \text{CHOH} \\
 & & \text{Erythritol.} & | & | \\
 & & & \text{CH}_2\text{OH} & \text{CHOH} \\
 & & & \text{Adonitol.} & | \\
 & & & & \text{CH}_2\text{OH} \\
 & & & & \text{Mannitol.}
\end{array}
$$
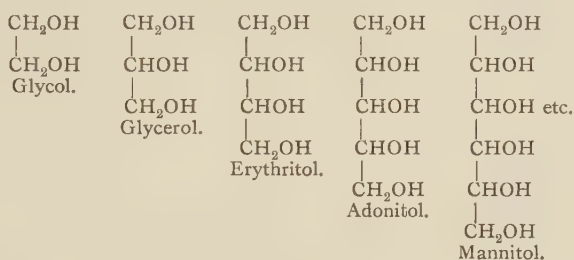
Glycol, the first member of the series, has been described (p. 136).

The trihydric alcohol, glycerol, is the chief member of the series, and is a constituent of fats. The other polyhydric alcohols are closely connected with the carbohydrates.

These compounds have the properties of primary and secondary alcohols.

Glycerol.

Glycerol occurs in nature in the free state. Small quantities are always present in beer, wine, and other fermented liquors. It is formed during the fermentation from the sugar. An increased amount of glycerol is produced if sodium sulphite, or bisulphite, is added to the fermenting solution. It occurs mainly in combination with fatty acids in the form of esters—the fats or glycerides—from which it is prepared by hydrolysis (p. 239) :—

$$
\begin{array}{ll}
\text{CH}_2\text{O—OC·R} & \text{CH}_2\text{OH} \\
| & | \\
\text{CHO—OC·R} + 3\text{H}_2\text{O} = \text{CHOH} + 3\text{HOOC·R} \\
| & | \\
\text{CH}_2\text{O—OC·R} & \text{CH}_2\text{OH}
\end{array}
$$
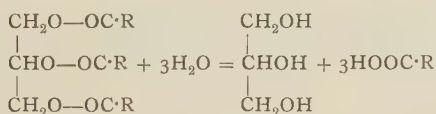
Preparation.

Glycerol is prepared from the aqueous solutions remaining after hydrolysis of fats, and removal of the fatty acids, or soaps (p. 239).

In the former case, the lime or sulphuric acid, is neutralised as calcium sulphate and the filtered solution evaporated *in vacuo*. In the latter case the separation is more difficult on account of the presence of salt. The raw glycerol is distilled *in vacuo*, or in superheated steam. By special condensing arrangements glycerol can be separated in a very concentrated state. Pure glycerol is then obtained by filtering through charcoal and removing the last traces of water *in vacuo*.

Properties.

Glycerol is a thick, colourless, very hygroscopic liquid without smell but with a sweet taste. It boils and distils under atmospheric pressure at 290° but undergoes slight decomposition; *in vacuo* it can be distilled without decomposition. If kept at 0° for some time, it crystallises and the crystals melt at 17°. It has a sp. gr. of 1·265 at 15°.

It mixes in all proportions with water and alcohol, but is insoluble in ether and chloroform.

Glycerol dissolves alkalies and many inorganic salts; its presence in a solution prevents the precipitation of cupric hydrate by alkalies. This behaviour is common to other compounds which contain several OH groups in their molecule, such as tartaric acid and the sugars.

The oily consistency of glycerol, its non-volatility at ordinary temperatures, its stability in the air, solvent power, sweet taste, and the property of making the skin soft and smooth are such useful and valuable properties that it is largely used in commerce and in various medicaments.

Reactions and Constitution.

Glycerol forms derivatives with sodium and other metallic hydroxides in which three hydrogen atoms are replaced by metals. It also forms esters with acids in which 1, 2, or 3 hydrogen atoms are substituted by acid groups. Phosphorus pentachloride replaces 1, 2, or 3 hydroxyl groups by chlorine. It therefore contains 3 hydroxyl groups.

On gentle oxidation it gives a mixture of glyceric aldehyde and dihydroxy acetone (see p. 249), and on further oxidation it gives firstly glyceric acid and secondly tartronic acid (p. 141), a dibasic acid. These

reactions show the presence of two primary alcohol groups, and a secondary alcohol group.

Synthesis.

The constitution of glycerol is proved by its synthesis :—

$$
\begin{array}{cccccc}
CH_3 & CH_3 & CH_2 & CH_2Cl & CH_2Cl & CH_2OH \\
| & | & \| & | & | & | \\
CO & \xrightarrow{H_2} CHOH & \xrightarrow{H_2SO_4} CH & \xrightarrow{Cl_2} CHCl & \xrightarrow{ICl_3} CHCl & \xrightarrow{H_2O} CHOH \\
| & | & | & | & | & | \\
CH_3 & CH_3 & CH_3 & CH_3 & CH_2Cl & CH_2OH \\
\text{Acetone.} & \text{Isopropyl} & \text{Propylene.} & \text{Propylene} & \text{Trichloro-} & \text{Glycerol.} \\
 & \text{Alcohol.} & & \text{Dichloride.} & \text{propane.} &
\end{array}
$$
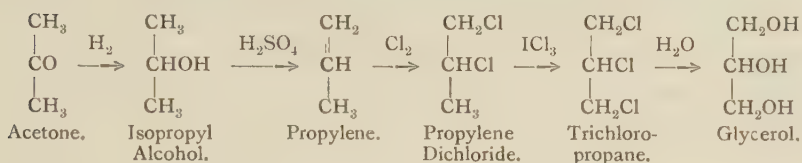
Tests.

* (1) On account of its oily appearance it may be mistaken for fat. On moistening a piece of paper with a drop of glycerol, the paper becomes marked as with a grease spot, but on washing the paper with water and drying, the spot is removed.

* (2) On heating a few drops of glycerol in a dry test tube with potassium bisulphate, or anhydrous phosphoric acid, the pungent odour of acrolein (p. 162) is noticed :—

$$CH_2OH . CHOH . CH_2OH = CH_2 : CH . CHO + 2H_2O.$$
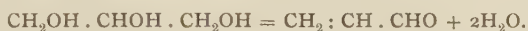
* (3) On adding an aqueous solution of glycerol (about 20 per cent.) to a 5 per cent. solution of borax, to which sufficient phenolphthalein solution has been added to produce a distinct red colour, the red colour is discharged, but on boiling it returns if excess of glycerol has not been used. This is known as Dunstan's test.

Any polyhydric alcohol may give this reaction. Ammonium salts also decolorise the solution, but the colour does not return on heating.

Esters of Glycerol.

Glycerol forms esters with 1, 2, or 3 molecules of acid. According to the position of the acid group in the molecule, two isomers are possible in the case of the mono- and di-esters :—

$$
\begin{array}{ccccc}
CH_2OAc & CH_2OH & CH_2OAc & CH_2OAc & CH_2OAc \\
| & | & | & | & | \\
*CHOH & CHOAc & *CHOAc & CHOH & CHOAc \\
| & | & | & | & | \\
CH_2OH & CH_2OH & CH_2OH & CH_2OAc & CH_2OAc \\
\underbrace{\qquad\qquad}_{\text{Mono-}} & & \underbrace{\qquad\qquad}_{\text{Di-}} & & \text{Tri-} \\
\alpha & \beta & \alpha, \beta & \alpha, \alpha &
\end{array}
$$
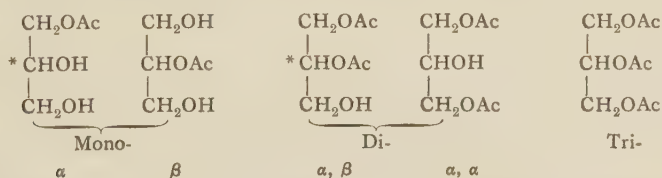
The middle carbon atom, marked with an asterisk in the α, and α, β esters is asymmetric ; these esters will exist in two stereoisomeric forms. The α, α ester and the tri-ester will exist in stereoisomeric forms, if the acid radicles in the molecule are different.

16

The chief esters are the trinitrate, the phosphoric, the tribenzoyl, and the fats (p. 236).

Glyceryl Trinitrate.   Nitroglycerine.

Glyceryl trinitrate, commonly known as nitroglycerine, is prepared by carefully dropping glycerol into a mixture of fuming nitric acid and concentrated sulphuric acid at 10°, and pouring into water.   Nitro-glycerine separates as an oil, which is freed from acids by washing with soda and water :—

$$
\begin{array}{ccc}
\mathrm{CH_2OH} & & \mathrm{CH_2 \cdot O-N\!\!<_O^O} \\[2pt]
| & & | \\[2pt]
\mathrm{CHOH} + \mathrm{3HO-N\!\!<_O^O} & = & \mathrm{CH \cdot O-N\!\!<_O^O} \\[2pt]
| & & | \\[2pt]
\mathrm{CH_2OH} & & \mathrm{CH_2 \cdot O-N\!\!<_O^O}
\end{array}
$$
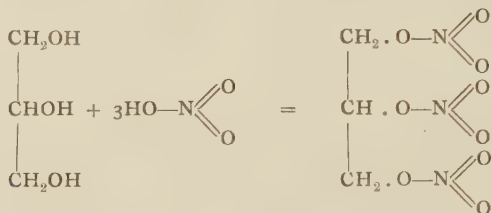
Nitroglycerine is an oily, colourless liquid without smell, with a sweet, later, burning taste.   It is scarcely soluble in water, but soluble in ab-solute alcohol and miscible with ether, chloroform, and benzene.   Its chief property is its ready explosiveness.   Heat alone will not cause nitroglycerine to explode.   A thin layer can be ignited and will burn quietly, but if suddenly heated to a low red heat, or struck, it detonates violently.   Dynamite, invented by A. Nobel, is a mixture of 75 per cent. nitroglycerine and 25 per cent. siliceous earth; the indifferent powder soaks up the oily nitroglycerine and gives a solid which can be transported without danger.   It is exploded by an electric spark, or by a percussion cap.

Glycerophosphoric Acid.

Two isomers are possible for the mono-phosphoric acid derivative :—

$$
\begin{array}{cc}
\mathrm{CH_2OPO(OH)_2} & \mathrm{CH_2OH} \\
| & | \\
\mathrm{CHOH} & \mathrm{CHOPO(OH)_2} \\
| & | \\
\mathrm{CH_2OH} & \mathrm{CH_2OH} \\
\alpha & \beta
\end{array}
$$
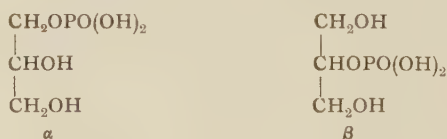
The α-glycerophosphoric acid will exist in two stereoisomeric forms. It is formed from lecithine by hydrolysis with baryta (p. 245).

Glycerophosphoric acid is prepared by heating glycerol with phos-phoric acid.   A mixture of the two forms results.

Glycerophosphoric acid is an acid syrup easily soluble in water.   It forms salts with metallic hydroxides.   The calcium and iron salts are used in medicine.   It is hydrolysed by acids into glycerol and phos-phoric acid.

Tetrahydric Alcohols.

Several polyhydric alcohols, containing 4 carbon atoms, the erythritols, exist. They contain two asymmetric carbon atoms and differ from one another in their stereochemistry (see tartaric acid). The inactive, internally compensated, compound occurs naturally in certain lichens.

Pentahydric Alcohols.

Five stereoisomeric forms of these polyhydric alcohols can exist. They have all been prepared by the reduction of the pentoses (p. 249) with sodium amalgam.

Adonitol occurs naturally in *Adonis vernalis*.

Hexahydric Alcohols.

These compounds contain four asymmetric carbon atoms and can exist in ten stereoisomeric forms.

They are closely related to the monosaccharides from which they are obtained on reduction and to which they give rise on oxidation. Mannitol, sorbitol, dulcitol, and iditol are found in nature.

Mannitol.

Mannitol is obtained from manna, the juice of the manna ash, by extracting it with hot water, or hot dilute alcohol, and crystallising the solution. It crystallises from water in prisms, from alcohol in silky needles, and melts at 165-166°. It requires 6 parts of water for solution; it is very slightly soluble in cold alcohol and almost insoluble in ether.

Sorbitol and iditol are present in the juice of mountain ash berries, and dulcitol is found in Madagascar manna.

CHAPTER XXVII.

FATS AND OILS. WAXES. LECITHINES OR LIPINES.

FATS and oils are present as reserve food material in most animal and vegetable tissues. They are the esters of the higher fatty acids, especially palmitic, stearic, and oleic acids, with glycerol. All these glyceryl esters, or glycerides, have similar names in which the name of the fatty acid describes the nature of the fat, e.g. butyrin, caproin, palmitin, olein.

$$\begin{array}{l} CH_2O-OC \cdot C_{17}H_{35} \\ | \\ CHO-OC \cdot C_{17}H_{35} \\ | \\ CH_2O-OC \cdot C_{17}H_{35} \end{array}$$
Stearin.
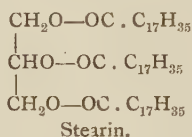
There is no chemical distinction between oils and fats; the solid esters are fats, the liquid esters are oils. The natural fats are mixtures of glycerides. The consistency of a fat, or oil, depends upon the nature of its constituents. Beef and mutton tallow which are hard solids contain chiefly palmitin and stearin. Lard and human fat which are soft solids contain more olein. Butter contains 4-6 per cent. of butyrin. Palm oil consists principally of palmitin. The vegetable oils, such as olive, cotton seed, and linseed oils contain chiefly olein, or other esters of unsaturated acids.

The oils are of two kinds; non-drying oils like olive oil and cotton seed oil; drying oils which oxidise in the air and become hard, like linseed oil. Hence its use for painting.

It is possible that the mixture of glycerides may in some cases be due to various fatty acids contained in the molecule, e.g.

$$\begin{array}{l} CH_2O-OC \cdot C_{17}H_{35} \\ | \\ CHO-OC \cdot C_{17}H_{33} \\ | \\ CH_2O-OC \cdot C_{15}H_{33} \end{array}$$
Palmito oleo-stearin.
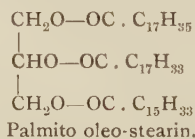
Fats are economically of great value as food, as illuminating agents, as lubricating agents, for soap and candle making, and for other purposes.

Properties.

Fats and oils have solubilities like the fatty acids. They are not soluble in water and are not soluble in dilute cold sodium hydroxide.

On warming, the fats melt and become oils. They have fairly definite melting-points. Fats and oils are not easily soluble in alcohol, but dissolve readily in ether, ligroin, carbon disulphide, etc.

Hardening of Fats.

Fats containing olein and other glycerides with unsaturated fatty acids can be reduced by hydrogen to the glyceride with the corresponding saturated fatty acid :—

$$
\begin{array}{ll}
CH_2O - OC . C_{17}H_{33} & CH_2O - OC . C_{17}H_{35} \\
| & | \\
CHO - OC . C_{17}H_{33} + 3H_2 = CHO - OC . C_{17}H_{35} \\
| & | \\
CH_2O - OC . C_{17}H_{33} & CH_2O - OC . C_{17}H_{35} \\
\text{Olein.} & \text{Stearin.}
\end{array}
$$
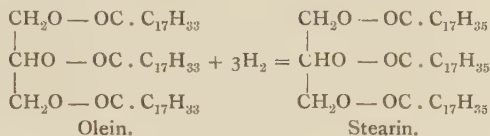
The oil becomes a solid fat and is "hardened." On a large scale this process is carried out in presence of nickel as a catalyst. The process of hardening of fats is used in the preparation of margarine from vegetable oils. Liquid fish oils can also be hardened by this means.

Extraction of Fats, etc.

The fats are contained in animal and vegetable tissues mixed with protein and carbohydrate. Three methods are in use for their separation. The oldest and simplest method consists in melting out the fat from the tissue by simply placing it in a suitable receptacle of muslin, or cloth, in a warm room ; the fat melts and runs out leaving the remainder of the tissue behind. The most modern method consists in pressing out the fat in a hydraulic press ; this is the method most frequently employed for obtaining the oils from vegetable seeds. The third method consists in extracting the fat by means of suitable solvents, such as benzine, carbon disulphide. Alcohol and ether are not usually employed for this purpose, but they are generally used if an estimation of fat in a tissue be required. For food the fat or oil is pressed out ; for soap making and other purposes the residue is extracted.

Estimation of Fats.

In the estimation of fat, the tissue must first be dried : this is effected by mixing a known weight of the material with clean dry sand, or other suitable absorbing medium, and then heating for 1-2 hours in a steam oven. In the case of milk, it is most convenient to absorb a known weight (or volume) in clean fat-free filter paper, which is made into a

small roll, and to dry this. The dried material is then placed in a paper thimble of suitable size and this is extracted with ether for 2-3 hours in a Soxhlet apparatus, which allows of a continual extraction for that time without constant attention.

The Soxhlet apparatus (Fig. 30) consists of (1) a small dry flask, the weight of which has been accurately determined, (2) a special extracting tube into which the thimble and material is placed, (3) a short condenser. The extracting tube is composed of a wide piece of glass tubing like a test tube fused at its closed end to a narrower piece of glass tubing which is cut off at an angle at its other extremity. Just below the join of these pieces of tubing a glass side tube is fused into the narrower piece; its other end is fused into the wider piece at the upper end. At the base of the wide tube, on the other side of the apparatus, one end of a narrow syphon tube is attached; its other end is fused to the narrow piece through which it passes and opens just above the angular extremity. The narrow end of this tube is fastened into the flask; the condenser is attached to the wider end. Ether is placed in the flask which is gently heated. The volatilised ether passes through the side tube and reaches the condenser. The condensed drops fall upon the thimble and cover it. When completely covered the ether is syphoned off and returns to the flask and the process is repeated. It remains to distil off the ether from the flask, dry at 100° and weigh. The difference in weight gives the amount of fat in the known weight of tissue.
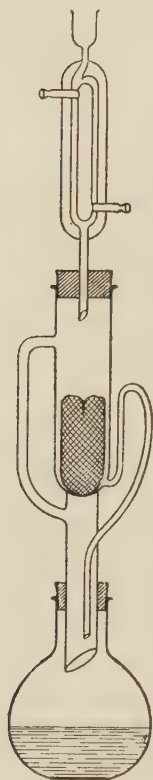
This method gives comparatively good results; other substances besides fats are extracted from the tissue and some of the fat, present inside the cells, is not extracted. It is now more usual in scientific investigations to estimate the fat as fatty acid.

FIG. 30.

Composition.   Hydrolysis.

Fats are hydrolysed into their constituent fatty acids and glycerol by boiling with water, treatment with steam, and by boiling with acids and alkalies. This last process of decomposing fats and esters is known as saponification and is a special form of hydrolysis; it was first used in the manufacture of soap, hence the term.

On the large scale, hydrolysis is effected (1) by heating under pressure with water and a small quantity of lime and then neutralising with sulphuric acid.   On cooling, the fatty acids set on the surface to a solid mass.   This mass is pressed to remove oleic acid and the remaining mixture of palmitic and stearic acids used for making candles.   (2) By heating with caustic soda in large open vessels.   Hard soap is produced ; it is separated by adding salt when it rises to the surface and sets into a solid mass on cooling.   (3) By heating with caustic potash.   Soft soap is produced ; it is usually not separated from the water and glycerol.

$$
\begin{array}{ll}
CH_2OOC . R & CH_2OH \\
| & | \\
CHOOC . R + 3KOH = CHOH + 3KOOC . R. \\
| & | \\
CH_2OOC . R & CH_2OH
\end{array}
$$
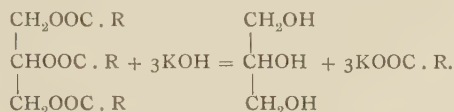
Another process of hydrolysing fats is by the action of the enzyme, lipase, contained in castor oil seeds.

Fats undergo the process of hydrolysis during digestion.   They are decomposed by the enzyme, lipase, in the pancreatic juice and hydrolysed into their constituents, fatty acids and glycerol.   Emulsification occurs in the intestine, where the reaction is alkaline, during the process of the hydrolysis.

In the laboratory with small quantities of fat, it is easier to effect the hydrolysis of fats by means of alcoholic soda.

(a) *Butter.*

A small quantity (2 gm.) of butter is heated with excess of alcoholic sodium hydroxide until a clear yellow solution is obtained.   No oil drops should be seen on pouring the solution into water.   The aqueous solution is heated to expel alcohol, acidified with dilute sulphuric acid, and again heated.   The smell of butyric and other volatile fatty acids is noticed.   They are obtained by distilling the acid solution.   The higher fatty acids are also present, but do not distil and remain as an oily layer on the surface of the hot liquid.

(b) *Olive Oil.*

If a little olive oil be dissolved in twice its quantity of ether and 5 times the volume of 2 per cent. alcoholic sodium hydroxide be added, and the mixture be allowed to stand in a corked vessel, it gradually solidifies and forms a jelly.   Complete saponification has occurred and soap has been formed.   The jelly dissolves in water and the soap solution will give (a) a precipitate of fatty acids on acidifying with sulphuric acid, and (b) a precipitate of the calcium soap on adding calcium chloride solution.

(c) Lard.

*   About 5 gm. of lard are boiled with 25 c.c. of 10 per cent. alcoholic sodium hydroxide under a reflux condenser for 5-10 minutes to saponify the fat.   25-50 c.c. of water are added ; if the saponification is complete, no oil drops should be seen ; if it be incomplete, the saponification is continued by adding alcoholic soda and again boiling.   The liquid is poured into an evaporating basin and the alcohol evaporated on a water-bath.   The solution is acidified with sulphuric acid ; the fatty acids are precipitated and are filtered off through a wet paper and washed free from acid with water.   The filtrate contains the glycerol which is detected as described below.

The presence of fatty acid in the precipitate is shown :—

*   (1) By dissolving a small portion in ether and adding the solution to alcohol containing a drop of phenolphthalein and a few drops of dilute sodium hydroxide.   The red colour disappears.

*   (2) By dissolving another portion in dilute sodium hydroxide.   A soap lather is formed on shaking it up with warm water.

The soap is salted out by adding sodium chloride and rises to the surface.

A precipitate of calcium soap is formed on adding calcium chloride.

*   (3) On heating with acid potassium sulphate there is no smell of acrolein, if the precipitate has been washed free from glycerol.

*   The presence of glycerol in the filtrate is shown by neutralising it and evaporating it to a syrup on the water-bath.   The syrup is mixed with alcohol which precipitates the sodium sulphate.   The alcoholic solution is poured off and evaporated, and the residue tested for glycerol by heating it with acid potassium sulphate, when acrolein is formed.

Analysis of Fats.

The natural fats consist of a mixture of the glyceryl esters of the saturated fatty acids, butyric, caproic, palmitic, and stearic, of the unsaturated fatty acids, oleic, linoleic and others and also of hydroxy fatty acids.   Free fatty acids are present in small quantities and increase in amount as the fat is kept.   The various natural fats and oils have a fairly constant composition so that by determining the amounts of the various constituents it can be identified.   The following six analyses are usually made :—

(1) the acid value, i.e. the amount of potassium hydroxide in mgm. required to neutralise the free fatty acid in 1 gm. of fat ;

(2) the saponification value, i.e. the amount of potassium hydroxide in mgm. required to saponify 1 gm. of the fat ;

(3) the iodine value, i.e. the amount of iodine in gm. absorbed by 100 gm. of the fat ;

(4) the Reichert-Meissl, or Reichert-Wollny, value, i.e. the amount of

potassium hydroxide in c.c. of ·1N required to neutralise the volatile fatty acids in 5 gm. of the fat;

(5) the Hehner value, i.e. the amount of non-volatile and insoluble fatty acids (and unsaponifiable matter) present in 5 gm. of the fat;

(6) the acetyl value, i.e. the amount of potassium hydroxide required to combine with the acetic acid in 1 gm. of fat, which has been acetylated.

The values of some of the commoner fats are given in the accompanying table :—

Fat.	Saponification value.	Iodine value.	Reichert-Wollny value.	Acetyl value.
Butter . .	220-233	26-50	26-33	2-8·6
Lard . .	195·5	46-70	·68	2-6
Tallow . .	192-200	35-46	0·5	2·7-8·6
Olive oil .	185-196	79-88	0·6	10·6
Linseed oil .	192-195	173-201	0	4·0
Cotton seed oil	193-195 .	108-110	—	—
Coco-nut fat .	246-260	8-10	6·6-7·0	1-12
Palm-nut fat .	242-250	13-17	5 6·8	2·8-5

Determination of the Acid Value.

A weighed amount of fat, from 3 or 5 to 10 gm., is dissolved in neutral alcohol,[1] or a mixture of alcohol and ether, a few drops of phenolphthalein are added and the solution is titrated with ·1N or ·5N potassium hydroxide until it is pink in colour. The pink colour should be permanent for about 2 minutes : after this time it usually disappears.

The acid value depends on the purity and age of the fat, i.e. on the amount of hydrolysis and oxidation that has taken place.

Determination of the Saponification Value.

The fat is saponified with an approximately ·5N solution of alcoholic potassium hydroxide, standardised against ·5N hydrochloric acid.

The alcoholic potassium hydroxide is prepared by dissolving 28 gm. of pure potassium hydroxide in a little water and diluting to 1000 c.c. with alcohol, or purified methylated spirit; after 24 hours the solution is filtered into a litre bottle, which is closed by a rubber stopper carrying a .25 c.c. pipette. The pipette is closed by a piece of rubber tubing and a glass rod.

In measuring out the volume it is convenient to allow the liquid to run out of the pipette without touching the sides of the vessel and then to let three drops fall from the end.

A weighed amount of fat (1·5 to 2 gm.) is heated in a 200 c.c. flask on a water-bath under a reflux condenser with 25 c.c. of the alcoholic potash solution for 30 minutes. At the same time a blank experiment (i.e. the same experiment without the fat) is carried out. The contents of the flasks are heated so that they boil gently and are shaken from time to time.

When the saponification is complete and the fat has dissolved giving a

[1] Methylated spirit which has been kept in contact with sodium hydroxide and distilled may be used. If it should contain acid it may be neutralised with ·1N potassium hydroxide until it shows a faint pink colour to phenolphthalein, a few drops of which are added as indicator.

clear solution, 1 c.c. of phenolphthalein solution is added and the mixture is titrated with ·5N hydrochloric acid.

The difference between the values found in the blank experiment and the one with fat is the volume of ·5N alkali required to saponify the fat. Hence the amount in mgm. required to saponify 1 gm. of fat can be calculated.

Determination of the Iodine Value.

There are two methods of determining the iodine value, (a) Hübl's, (b) Wijs'. The process is the same in the two methods, the difference being in the iodine solution. Hübl used a mixture of iodine and mercuric chloride, Wijs iodine trichloride.

The following reagents are required :—

(1) Iodine solution.

(a) Hübl's. Equal volumes, e.g. 30 c.c. of a solution of 25 gm. of iodine in 500 c.c. of pure 95 per cent. alcohol and of a solution (filtered if necessary) of 30 gm. of mercuric chloride in 500 c.c. of pure 95 per cent. alcohol are mixed 12-24 hours before use. The mixture should not be used if it has been prepared longer than 24 hours.

(b) Wijs. 9·4 gm. of iodine trichloride are dissolved in 200 c.c. of glacial acetic acid contained in a 300 c.c. flask heated on the water-bath and closed with a cork carrying a calcium chloride tube ; at the same time 7·2 gm. of finely powdered iodine are dissolved in glacial acetic acid in a similar way. The two solutions are poured into a 1000 c.c. flask and any undissolved iodine dissolved in a fresh portion of acetic acid. The mixed solutions are cooled and made up to 1000 c.c.

This solution may also be made by dissolving 13 gm. of iodine in 1000 c.c. of glacial acetic acid and passing washed and dry chlorine through the solution. A colour change occurs at the point when iodine trichloride is formed. In preparing the solution, the iodine content of the solution is determined before the passage of the chlorine ; the chlorine is passed into the solution until the iodine content is doubled (Lewkowitch).

(2) ·1N thiosulphate solution. This is prepared by dissolving 24·823 gm. of the pure salt in 1000 c.c. of water, or by dissolving 25 gm. of the salt in 1000 c.c. and standardising after 24 hours against potassium bichromate as follows :—

20 c.c. of a bichromate solution containing 3·8657 gm. in 1000 c.c. are placed in a bottle containing 10 c.c. of the potassium iodide solution. ·5 c.c. of concentrated hydrochloric acid are added. The brown solution is titrated with the thiosulphate solution, using starch solution as indicator.

Since 20 c.c. of the bichromate solution yields 0·2 gm. iodine, 1 c.c. of

thiosulphate $= \dfrac{\cdot 2}{x}$ gm. of iodine, if x c.c. are required in the titration.

(3) 10 per cent. potassium iodide solution. The quantity of iodate present must be taken into consideration.

(4) 1 per cent. starch solution.

(5) Pure chloroform, or carbon tetrachloride. Their purity is tested by adding 10 c.c. of iodine solution and titrating after 2-3 hours. The value should be same as for 10 c.c. iodine solution.

Pure glacial acetic acid (purified by recrystallisation). It should not give a green colour on heating with bichromate and sulphuric acid after prolonged standing.

Procedure :—

·15 to ·18 gm. of marine animal oil, ·2 to ·3 gm. of semi-drying oil, ·3 to ·4 gm. of non-drying oil, or ·8 to 1·0 gm. of solid fat is placed in a 500-800 c.c. bottle with well-fitting stopper and dissolved in 10 c.c. of chloroform, or carbon tetrachloride.[1]   25 or 50 c.c. of iodine solution are added, the pipette being allowed to drain for 2 or 3 drops after it has emptied.   Iodine solution and solvent must give a clear solution on shaking, otherwise more solvent is added.   The mixture is kept in a dark place for 6-8 hours in the case of fat and non-drying oils, for 8-10 hours in the case of semi-drying oils, or for 12-18 hours in the case of marine animal oils.   The mixture must contain 3 times the amount of iodine necessary and should be deep brown after 2 hours. More iodine solution is added, if the colour be paler.   15-20 c.c. of potassium iodide solution and 400 c.c. of water are added and the iodine titrated with ·1N thiosulphate.   If a red precipitate should form, more potassium iodide must be added.   25 c.c. of the iodine solution are titrated previously, or subsequently.   The difference gives the amount of iodine absorbed.   The result is calculated :—

$$
\left.
\begin{array}{l}
\text{e.g. 25 c.c. iodine solution} = 35\cdot5 \text{ c.c. } \cdot1\text{N Thio} \\
0\cdot983 \text{ gm. fat } + 25 \text{ c.c. iodine solution} = 18\cdot6 \text{ c.c. } \cdot1\text{N Thio}
\end{array}
\right\}
\begin{array}{l}
\therefore \text{ iodine used } = 16\cdot9 \text{ c.c. } \cdot1\text{N} \\
\quad\text{,, \quad ,, \quad} = 16\cdot9 \times \cdot0127 \\
\quad\quad\quad\quad\quad\quad\quad \text{gm. iodine}
\end{array}
$$

$$\therefore \text{ 100 gm. fat} = 21\cdot8 \text{ gm. iodine.}$$

Determination of the Reichert-Wollny Value.

The standard apparatus for this estimation is shown in Fig. 31.

<p style="text-align:center">FIG. 31.</p>

5 gm. of the fat are weighed out into a flask with a flat bottom of about 250 c.c. capacity and with a neck 2 cm. wide and 7-8 cm. long.   2 c.c. of a solution of 98 per cent. caustic soda in an equal weight of water, which is protected from carbonic acid, and 10 c.c. of 92 per cent. alcohol are added. The contents are heated on a water-bath under a reflux condenser for 15 minutes ; the alcohol is removed by warming the flask for about 30 minutes

[1] Glacial acetic acid in the case of oxidised oils not completely soluble in carbon tetra-chloride.

without a condenser; 100 c.c. of boiling water, freed from carbon dioxide by boiling for at least 10 minutes, are added and the soap dissolved. The flask is connected to a condenser by a bent tube 7 mm. in diameter and 15 cm. in length from the cork of the flask. The condenser is 8 mm. in diameter and 35 cm. long. The contents of the flask are acidified with 40 c.c. of N sulphuric acid, a piece of porcelain added, and distilled from an asbestos board 12 cm. in diameter containing an opening 5 cm. in diameter. The heating is begun cautiously so as to melt the fatty acids and then at such a rate that 110 c.c. distil over in about 30 minutes. The distillate is collected in a measuring flask of 110 c.c. capacity. The distillate is mixed and 100 c.c. are titrated with ·1N alkali, using 0·5 c.c. of 1 per cent. phenolphthalein solution as indicator.

Determination of the Hehner Value.

3-4 gm. of the fat are weighed out in a porcelain basin 13 cm. in diameter; 50 c.c. of alcohol and 1-2 gm. of potassium hydroxide are added. The mixture is heated on a water-bath with constant stirring till saponification is complete and until a clear solution is obtained. If a drop of water be added and no turbidity be produced, the saponification is complete. The solution is evaporated till it becomes pasty and 100-150 c.c. of water are added. It is acidified with sulphuric acid and warmed till the fatty acids form an oily layer on the surface. The fatty acids are filtered off on to a weighed filtered paper 10 cm. in diameter. This should have a texture so that it prevents fatty acid from passing through it and it is half filled with hot water and kept at this level with hot water during the filtration. The fatty acids are washed till the washings no longer react acid. The filter and its contents are dried at 100° for 2 hours, cooled, and weighed.

Determination of the Acetyl Value.

10 gm. of the fat are boiled with twice the weight of acetic anhydride in a round flask under a reflux air condenser for 2 hours. The solution is poured into about 500 c.c. of hot water contained in a beaker and boiled for half an hour, whilst a slow current of carbon dioxide is passed through it to prevent bumping. On standing, it separates into two layers; the aqueous layer is syphoned off and the remaining oil washed 3 times with water, so as to remove acetic acid, which may be tested for by its acid reaction. The acetylated fat is collected on a filter paper and dried at 100°.

2-5 gm. of the acetylated product are saponified with ·1N alcoholic potash as described under determination of the saponification value. The solution is evaporated to expel the alcohol and the residue dissolved in water. The same volume of ·1N acid as of alkali used in the saponification is added and the solution warmed. The aqueous solution is syphoned off through a wet filter and the fatty acids washed with hot water till all the acid is removed. The filtrate and washings are titrated with ·1N alkali using phenolphthalein as indicator. Soluble fatty acids, if present in the fat, must be separately determined in the same way and their amount deducted from the value obtained.

Waxes.

Waxes, which in appearance somewhat resemble fats, are chemically very different. They are esters of the higher alcohols, cetyl alcohol, $C_{16}H_{33}OH$, myristic alcohol, $C_{30}H_{61}OH$, and cholesterol, $C_{27}H_{45}OH$, with the higher fatty acids, e.g. :—

Carnauba wax contains ceryl and myricyl alcohols and cerotic and carnaubic acids.

Wool wax, or lanolin, contains cholesterol.

Spermaceti is the palmitic acid ester of cetyl alcohol.

Waxes are hydrolysed by means of alcoholic soda and the solution is diluted with water. The alcohol, which is insoluble in water, is extracted with ether and is obtained on evaporation. The alkaline solution on acidification gives a precipitate of the fatty acids.

Lecithines, or Lipines.   Cerebrosides.

Lecithines.

In all animal and vegetable cells, intimately associated with the life processes and existing in loose combination with protein, there are substances having the general composition and properties of fat, but containing in addition phosphoric acid, and choline, or amino-ethyl alcohol.   They are lecithine and kephaline :—

$$
\begin{array}{ll}
\text{CH}_2\text{O—OCR} & \text{CH}_2\text{O—OCR} \\
\text{CHO—OCR}' & \text{CHO—OCR}' \\
\text{CH}_2\text{O—OP}\big\langle{}^{\text{OH}}_{\text{O—CH}_2 \,.\, \text{CH}_2\text{N(CH}_3)_3\text{OH}} & \text{CH}_2\text{O—OP}\big\langle{}^{\text{OH}}_{\text{O}\,.\,\text{CH}_2\,.\,\text{CH}_2\,.\,\text{NH}_2} \\
\qquad\text{Lecithine.} & \qquad\text{Kephaline.}
\end{array}
$$
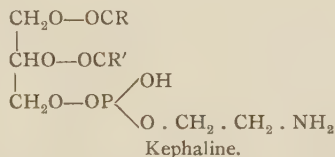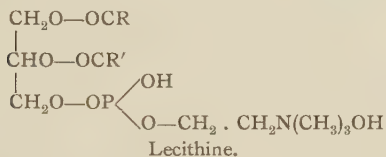
These compounds form a group called the lecithines, or lipines. The two lipines are generally found together in most tissues and are difficult to separate. The fatty acids in the molecule are a saturated acid, and an unsaturated acid.

About 10 per cent. of lecithine is present in egg-yolk ; liver and blood contain about 2 per cent. Heart tissue, kidney tissue also contain considerable quantities. Vegetable tissues contain ·25-1·5 per cent.

Lecithine is most usually extracted from dried tissues by solution in cold alcohol. The alcoholic solution is evaporated and the residue dissolved in ether. Lecithine is precipitated by the addition of acetone.

Lecithine is converted into its constituents, fatty acids, choline, and glycerophosphoric acid by hydrolysis, usually by means of alcoholic soda. The solution is diluted with water and acidified with sulphuric acid. The fatty acids are precipitated and extracted with ether. The aqueous solution is neutralised and evaporated to dryness. The dry residue is treated with alcohol which dissolves the choline and amino-ethyl alcohol, leaving the glycerophosphate. Choline may be precipitated by mercuric chloride, and amino-ethyl alcohol by gold chloride.

Lecithine has the appearance of a soft fat, generally brown in colour. It is easily soluble in cold absolute alcohol, in ether, and other organic solvents, but is nearly insoluble in acetone. It can therefore be precipitated from a concentrated solution in ether by the addition of acetone. Kephaline is not soluble in alcohol, but otherwise resembles lecithine very closely.

Sphingomyeline.

The substance, sphingomyeline, is also found in tissues, but in larger quantities in brain substance. It contains phosphorus like lecithine and kephaline, but not glycerol. Its constituents are two fatty acids, choline, sphingosine, and phosphoric acid. Sphingosine is an unsaturated compound and contains two hydroxyl groups :—

$$C_{12}H_{25} . CH=CH . CHOH . CHOH . CH_2 . NH_2.$$

Cerebrosides.

Brain tissue contains sphingomyeline and the two cerebrosides, phrenosine and kerasine. These three substances form the group of phosphatides. The mixture constitutes protagon. The cerebrosides do not contain phosphorus. They are composed of the base sphingosine, galactose, and a fatty acid, the sphingosine in combination with the galactose in the form of a glucoside and in combination with the fatty acid in the form of an acid amide :—

galactose—sphingosine—phrenosinic acid ($C_{25}H_{50}O_3$).
‾‾
phrenosine.
galactose—sphingosine—lignoceric acid ($C_{24}H_{48}O_2$).
‾‾
kerasine.
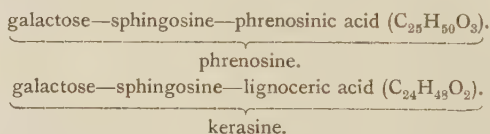
These compounds have also been isolated from other organs.

The cerebrosides dissolve in hot alcohol, acetone, benzene, but not in the cold solvent. They are insoluble in ether.

A full account of these substances is given in Maclean's monograph on lipines.

CHAPTER XXVIII.

THE CARBOHYDRATES.

THE carbohydrates are a large group of compounds especially abundant in plants ; the amount present in animals is by comparison very small. In appearance the carbohydrates are very different. Many are easily soluble in water, have a sweet taste, and are easily crystallised—these are the sugars. Others are insoluble in water, without any characteristic taste and have not been obtained in a crystalline form. Such compounds are starch, cellulose, and gums. The latter are very complex and form the structural basis of plants. Starch and the sugars are deposited in various parts as reserve material and as food-stuffs for the young plant. The starches and the sugars are the chief food-stuffs of animals.

The empirical formula of the carbohydrates shows that they consist essentially of carbon and water, $C_m(H_2O)_n$— hence their name—though substances other than carbohydrates, e.g. formaldehyde, CH_2O, acetic acid, $C_2H_4O_2$, and lactic acid, $C_3H_6O_3$, also possess the same empirical formula, and some carbohydrates have empirical formulæ in which the ratio of the elements $H : O$ is not $2 : 1$, e.g. the methyl pentose, rhamnose $C_6H_{12}O_5$.

The group of carbohydrates contains simple and complex members. The simple members contain 2, 3, 4, 5, 6, 7, 8, 9 carbon atoms in their molecule, the chief physiological representatives being the members with six carbon atoms, and in a lesser degree those with 5 atoms of carbon. It was formerly supposed that only those members containing 6 atoms of carbon belonged to the class of sugars, and it is convenient to term the six carbon atom representatives the sugars, whilst the whole group is termed the carbohydrates. They are essentially compounds of the nature of alcohols, primary and secondary, and at the same time aldehyde, or ketone.

The complex members consist of combinations together in an anhydride form of 2, 3, 4 and more of the simple units, generally of

those containing 6 carbon atoms, sometimes of those with 5 carbon atoms. Accordingly as they contain 2, 3, etc., simple units in combination they are termed disaccharides, trisaccharides, or polysaccharides, the simple unit being termed a monosaccharide.

All the complex members are converted into their constituent single units by hydrolysis with acids.

The members of the carbohydrate group are distinguished by the Greek numeral to indicate the number of carbon atoms in the molecule and by the suffix -ose. According to the presence of an aldehyde, or ketone, group, they may be aldoses, or ketoses. Thus, aldotriose, ketohexose. The suffix -ose is not applied to many of the complex compounds.

As the physical properties, appearance, solubility, taste, etc., of the various carbohydrates is very different, no proper classification can be based upon these properties. They are classified according to their complexity :—

Monosaccharides.

Diose.	Glycollic aldehyde.
Trioses.	Glyceric aldehyde, dihydroxyacetone.
Tetroses.	Erythrose, threose.
Pentoses.	Arabinose, xylose, ribose, etc.
Hexoses.	Glucose, mannose, galactose, etc.
	In this group it is convenient to include *d*-glucosamine, or aminoglucose.
	Fructose, sorbose, etc.
Heptoses, etc.	

Disaccharides.
Sucrose, maltose, lactose, etc.

Trisaccharides.
Raffinose.

Tetrasaccharides.
Stachyose.

Polysaccharides.
Starch, cellulose, dextrin, glycogen, inulin.
Gums, pectins, pentosans, mannosans, etc.

THE MONOSACCHARIDES.

Diose.

Glycollic aldehyde, $\begin{array}{c} CH_2OH, \\ | \\ CHO \end{array}$ which contains a primary alcohol group and an aldehyde group, is the first member of the series. It is derived, like all the members of the carbohydrate group, by oxidation of the corresponding dihydric alcohol, glycol.

Trioses. Glyceroses.

Glyceric aldehyde and dihydroxyacetone are obtained from gly-

$$\begin{array}{ccc} CH_2OH & & CH_2OH \\ | & & | \\ CHOH & & CO \\ | & & | \\ CHO & & CH_2OH \end{array}$$
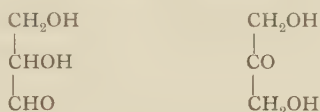
cerol by oxidation with sodium hypobromite, or hydrogen peroxide in the presence of ferrous sulphate. With hydrogen peroxide, glyceric aldehyde is the chief product, with sodium hypobromite, dihydroxy-acetone. They contain respectively an aldehyde and ketone group and they are the first examples of an isomeric aldose and ketose. Glyceric aldehyde contains an asymmetric carbon atom, but the two stereoisomers, d- and l-glycerose, have not yet been separated.

Both compounds have been obtained in crystalline form. They have a sweet taste and generally have the properties of an aldehyde, or ketone. The presence of the hydroxyl groups in dihydroxyacetone causes reduction of Fehling's solution.

Tetroses.

The tetroses contain 4 atoms of carbon. An aldose and a ketose are theoretically possible. The aldoses contain two asymmetric carbon atoms. Four stereoisomers of these aldotetroses, d- and l-erythreose, d- and l-threose are known. The ketose, erythrulose, has one asymmetric carbon atom and exists in d-, l-, and dl-forms.

Pentoses.

Five carbon atoms are present in the molecule of a pentose, and isomers, an aldose and 2 ketoses, are possible. Three asymmetric carbon atoms are present: 2^3 or 8 stereoisomeric aldoses can exist. All but one are known, but all do not occur in nature.

d-ribose is contained in the nucleic acid of plants from which it is obtained by hydrolysis.

l-arabinose is contained in the polysaccharides cherry gum, gum arabic, peach gum. . It is obtained by the hydrolysis of these substances with dilute sulphuric acid. Arabinose crystallises in prisms, has a sweet taste, is dextrorotatory, though termed *l*-arabinose on account of its stereochemical relation to glucose; it melts at 160°.

Xylose is obtained by the hydrolysis of wood gum or xylane, straw, and various forms of cellulose. It is optically inactive and melts at 144-145°.

A pentose, as yet not definitely identified, but probably arabinose, or ribose, is excreted in the urine in certain diseases.

Methyl Pentoses.

Rhamnose, $C_6H_{12}O_5$, is obtained by the hydrolysis of the glucosides, quercitrin, xanthorhamnin, and some saponins. Rhamnose crystallises with a molecule of water, $C_6H_{14}O_6$, and was formerly regarded as a hexahydric alcohol—isodulcitol. It melts at 93°.

Fucose in seaweed, chinovose in chinovin, and other methyl pentoses have also been prepared.

HEXOSES.

The hexoses contain 6 atoms of carbon; two isomeric ketoses and one aldose are possible. Four asymmetric carbon atoms are present in the molecule of an aldohexose; 2^4 or 16 stereoisomers are possible, but only three are found in nature. Most of the other stereoisomers have been prepared in the laboratory by Emil Fischer. Two stereoisomeric ketoses are also found in nature. The formulæ of the natural hexoses are :—

CHO	CHO	CHO	CH$_2$OH	CH$_2$OH	1
H.C.OH	HO.C.H	H.C.OH	CO	CO	2
HO.C.H	HO.C.H	HO.C.H	HO.C.H	HO.C.H	3
H.C.OH	H.C.OH	HO.C.H	H.C.OH	H.C.OH	4
H.C.OH	H.C.OH	H.C.OH	H.C.OH	HO.C.H	5
CH$_2$OH	CH$_2$OH	CH$_2$OH	CH$_2$OH	CH$_2$OH	6
d-glucose.	*d*-mannose.	*d*-galactose.	*d*-fructose.	*d*-sorbose.	
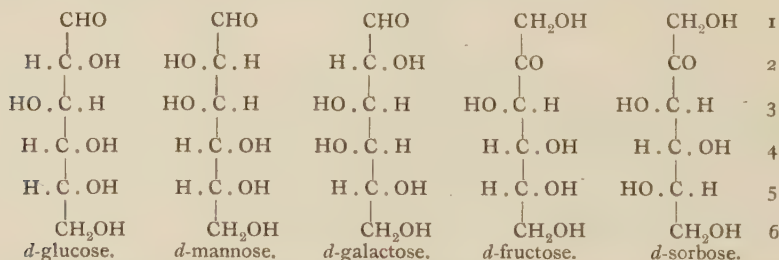
The difference between the aldehexoses is due only to stereoisomerism. The two keto-hexoses also differ only in stereoisomerism. It is customary to number the carbon atoms, starting from the fixed aldehyde group at the end of the chain. It will be noticed that *d*-glucose and *d*-mannose differ only in the arrangement of the groups upon carbon atom 2; *d*-galactose differs from *d*-glucose in the arrangement upon carbon atom 4. Except for the ketone group in *d*-fructose, *d*-glucose and

d fructose have the same structure.   In fact, *d*-glucose, *d*-mannose and *d*-fructose are very closely related, and are easily converted into each other (see below).   They are the only sugars easily fermented by yeast to alcohol and carbon dioxide.

Constitution of the Aldoses (Glucose).

Analysis and molecular weight determinations show that glucose has the empirical formula $C_6H_{12}O_6$.   The 6 atoms of carbon are joined together in a straight chain as is shown by its reduction with hydriodic acid into normal hexyl iodide,

$$CH_3 . CH_2 . CH_2 . CH_2 . CHI . CH_3.$$

The formation of esters with 5 molecules of acid show the presence of five OH groups.   These must be attached to different carbon atoms.

* Thus, pentabenzoyl glucose is precipitated when a solution of glucose is shaken with benzoyl chloride and excess of sodium hydroxide :—

$$C_6H_7O(OH)_5 + 5C_6H_5 . CO . Cl = C_6H_7O(O . OC . C_6H_5)_5 + 5HCl.$$

The remaining group is an aldehyde group as shown by the aldehyde reactions and the formation of an acid, gluconic acid, with the same number of carbon atoms by oxidation with bromine water.   Further oxidation of glucose by means of nitric acid gives a dibasic acid which must arise by the oxidation of a primary alcohol group.   The addition of hydrogen cyanide and hydrolysis of the cyanohydrin to normal heptylic acid confirms the constitution of glucose.

Glycuronic acid, another oxidation product of glucose, is formed in the animal body ; in the formation of this compound the primary alcohol group of glucose is oxidised whilst the aldehyde group is unchanged.   Glycuronic acid is formed under certain conditions, e.g. after the administration of chloral hydrate.   Chloral, and also other compounds, apparently combine with the aldehyde group of glucose ; the alcohol group is then oxidised in the body and the combination product (paired glycuronic acid) is excreted in the urine.   The best source of glycuronic acid is euxanthic acid, the calcium and magnesium salt of which constitutes the yellow pigment, Indian yellow.   This is obtained from the urine of cows that have been fed on mango leaves.   Glycuronic acid is prepared by hydrolysis of the Indian yellow, or other combination product.   We have therefore

CH_2OH	CH_2OH	$COOH$	$COOH$
$(CHOH)_4$	$(CHOH)_4$	$(CHOH)_4$	$(CHOH)_4$
CHO	$COOH$	$COOH$	CHO
Glucose.	Gluconic acid.	Saccharic acid.	Glycuronic acid.
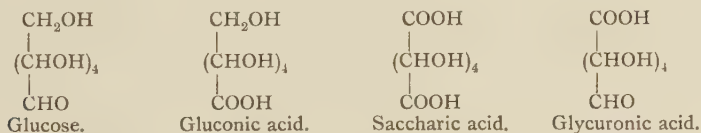
Constitution of the Ketoses (Fructose).

The 6 carbon atoms in fructose are present in a straight chain, as it can be reduced to normal hexyl iodide.   Five hydroxyl groups are

present, as it forms esters with 5 molecules of acid. On oxidation, fructose breaks down giving trihydroxybutyric acid and glycollic acid, which shows the presence of a ketone group and in the position shown in the formula. Further proof of the position of the ketone group is given by the formation of a cyanohydrin which yields an acid. This acid on hydrolysis gives methylbutylacetic acid.

The other hexoses behave exactly like glucose and fructose. They differ only in their stereoisomerism.

Glucose and the other hexoses are represented above as hydroxy-aldehydes, or ketones, but this constitution does not entirely explain all their chemical and physical properties.

(1) Glucose in its chemical behaviour is less active than is expected.

(2) A freshly prepared solution of glucose shows a higher rotatory power than a solution which has been kept for some hours (Muta-rotation). Tanret has isolated from glucose solutions, under certain conditions, two compounds, one of which, a-glucose, has a high initial rotatory power of 110°, the other, β-glucose, a low one of 19°. On being kept in solution both compounds give a solution of the same rotatory power of 52·5°.

(3) Two isomeric methyl, etc., derivatives (glucosides) are also obtainable from glucose.

These properties are most satisfactorily explained by assuming that glucose and also the other hexoses have formulæ in which four or five carbon atoms and an oxygen atom are included in a ring. They are derived from the hypothetical aldehydrol by loss of water which may occur at other parts of the molecule than at the aldehyde grouping :—

CHO	CH〈OH〈OH	CHOH	CHOH	CHOH	CHOH
CHOH	CHOH	CH〉O	CHOH ∖	CHOH ∖	CHOH
CHOH →	CHOH →	CHOH or	CH 〉	CHOH O	or CHOH ∖ O
CHOH ←	CHOH ←	CHOH	CHOH	CH ∕	CHOH ∕
CHOH	CHOH	.CHOH	CHOH	CHOH	CH ∕
CH₂OH	CH₂OH	CH₂OH	CH₂OH	CH₂OH	CH₂OH
Aldehyde form.	Aldehydrol form.	Ethylene oxide form.	Propylene oxide form.	Butylene oxide form. γ-lactone.	Amylene oxide form.
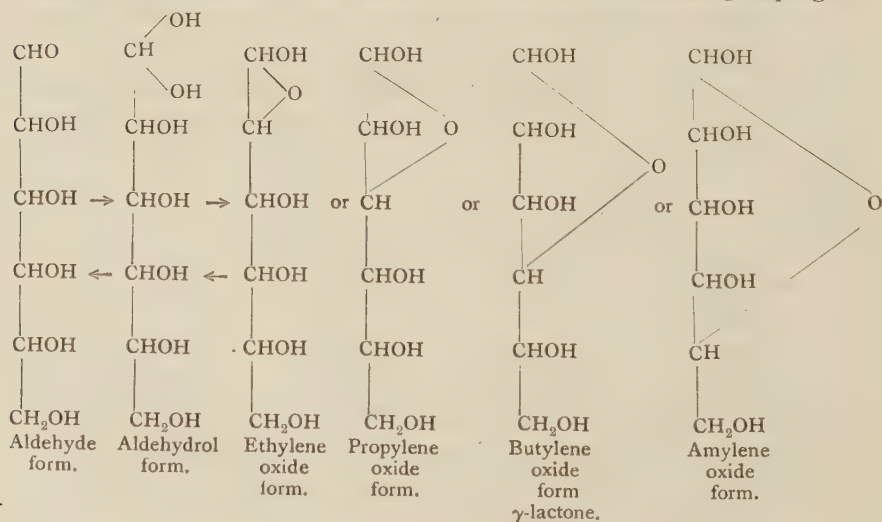
Of these various forms the butylene oxide, or γ-lactone, form has generally been regarded as the most probable formula for glucose, but the amylene

oxide form is considered by Haworth (1925) to be the correct formula. The presence of all, or any of these, forms in solution is easily possible, as the above changes are reversible. If glucose is acting as true aldehyde and is being removed, some of the other form will change to aldehydrol and to aldehyde, and *vice versa* if some of the oxide form is removed by reaction, more is formed from aldehyde. It is also possible for butylene oxide to change to aldehydrol and then to propylene oxide, or amylene oxide.

This change of oxide form to aldehyde takes time and accounts for the slower activity of glucose.

If glucose has an oxide formula, carbon atom 1 becomes asymmetric, and, irrespective of the stereoisomerism due to the other four carbon atoms, two possible compounds should exist. Their existence is shown (1) by the isolation of α-glucose and of β-glucose, and (2) by the formation of two derivatives, or glucosides, from glucose. The two stereoisomeric forms are :—

H . C . OH   HO . C . H
| |
CHOH   CHOH
| |
CHOH   O   and   CHOH   O
| |
CHOH   CHOH
| |
CH   CH
| |
CH_2OH CH_2OH
α-glucose.   β-glucose.
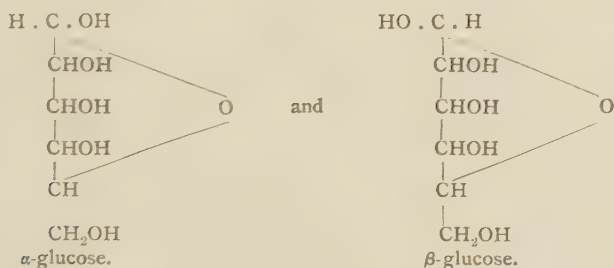
and they give rise to the two glucosides.

The existence of several possible oxide forms accounts for another variety of glucose, called γ-glucose, which is much more reactive than ordinary glucose. This γ-glucose may have the ethylene oxide formula. It is believed that the change to γ-glucose is necessary for the catabolism of glucose in the body. If the change of ordinary glucose to γ-glucose is not effected, glucose will not be oxidised in the body and will accumulate in the blood and tissues and be excreted in the urine. Such is the condition in diabetes ; the body cannot catabolise glucose, possibly because the mechanism to change it to γ-glucose is lacking.

The oxide form applies also to mannose and galactose and to fructose, which will be represented by

CH_2OH
| OH
C
|
CHOH
| O
CHOH
|
CH
|
CH_2OH
Fructose.
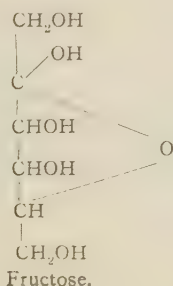
Interconversion of Glucose, Fructose and Mannose.

Solutions of glucose in presence of dilute alkali are slowly changed into solutions containing fructose and mannose.   Similarly, fructose and mannose are converted into a mixture of the other 2 hexoses.   An equilibrium condition is reached in which all 3 hexoses are present· This interconversion probably takes place through a change of the aldehyde, or ketone, form to a keto-enol form, which may then become ketone, or other aldohexose : —

$$
\begin{array}{ccccc}
\text{CHO} & & \text{CHOH} & & \text{CH}_2\text{OH} \\
| & & \| & & | \\
\text{CHOH} & & \text{C·OH} & & \text{CO} \\
| & & | & & | \\
\text{CHOH} & \rightarrow & \text{CHOH} & \rightarrow & \text{CHOH} \\
| & & | & & | \\
\text{CHOH} & \leftarrow & \text{CHOH} & \leftarrow & \text{CHOH} \\
| & & | & & | \\
\text{CHOH} & & \text{CHOH} & & \text{CHOH} \\
| & & | & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
\text{Glucose} & & \text{Keto-} & & \text{Fructose.} \\
\text{or} & & \text{enol} & & \\
\text{Mannose.} & & \text{form.} & &
\end{array}
$$
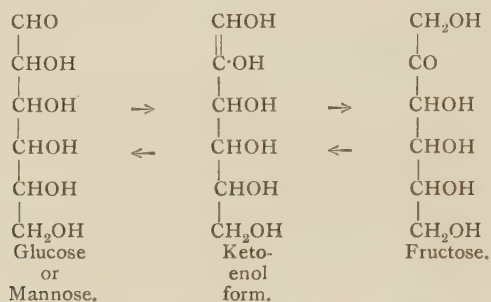
Reference to the stereoisomerism of these 3 hexoses shows that the only difference is upon carbon atom 2.   If the keto-enol form changes to aldehyde form, there is the formation of either, or both, glucose and mannose.

The conversion of fructose to mannose can be easily effected chemically.   On reduction, fructose gives sorbitol and mannitol. Mannitol, on oxidation, gives mannose.

The conversion of glucose to mannose and of mannose to glucose is effected chemically by oxidation to gluconic acid and mannonic acid respectively.   If these acids are heated with pyridine, or quinoline, they are converted from gluconic acid to mannonic acid and *vice versa*.   Reduction of the acids gives glucose, or mannose.

The conversion of glucose to fructose is effected through the glucosazone (see below).

Synthesis and Degradation of Glucose.

In studying the chemistry of the hexoses, it has been necessary to prepare hexoses from pentoses, and to degrade hexoses to pentoses.   The synthesis of hexose from pentose is effected by the cyanhydrin reaction :—

$$
\begin{array}{ccccccc}
 & & \text{CH}\big<^{\text{CN}}_{\text{OH}} & & \text{COOH} & & \text{CHO} \\
\text{CHO} & & | & & | & & | \\
| & & | & & \text{CHOH} & & \text{CHOH} \\
(\text{CHOH})_3 & \rightarrow & (\text{CHOH})_3 & \rightarrow & (\text{CHOH})_3 & \rightarrow & (\text{CHOH})_3 \\
| & & | & & | & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
\text{Pentose.} & & \text{Cyanhydrin.} & & \text{Hexonic acid.} & & \text{Hexose.}
\end{array}
$$
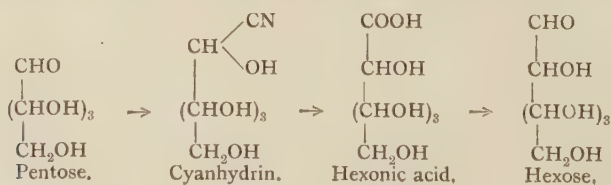
Glucose can thus be converted to a heptose, the heptose to an octose, and the octose to a nonose.   The particular hexose, which results, depends on the pentose at the start.

Several methods for changing a hexose to a pentose have been discovered. The simplest are the following :—

(1) Glucose is converted into its oxime with hydroxylamine.   On heating the oxime with conc. hydrochloric acid, it gives the nitrile of gluconic acid which loses hydrogen cyanide on further heating and gives a pentose :—

$$
\begin{array}{cccc}
\text{CHO} & \text{CH : NOH} & \text{CN} & \\
| & | & | & \\
\text{CHOH} & \text{CHOH} & \text{CHOH} & \text{CHO} \\
| & | & | & | \\
\text{(CHOH)}_3 & \text{(CHOH)}_3 & \text{(CHOH)}_3 & \text{(CHOH)}_3 \\
| & | & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
\end{array}
$$
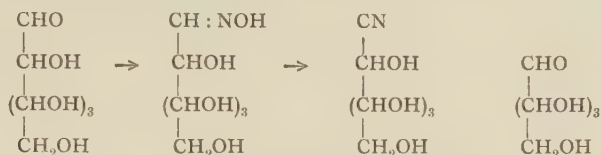
(2) Glucose is oxidised to gluconic acid ; gluconic acid, on oxidation with hydrogen peroxide in the presence of ferrous salts, yields a pentose :—

$$
\begin{array}{ccc}
\text{CHO} & \text{COOH} & \\
| & | & \\
\text{CHOH} & \text{CHOH} & \text{CHO} \\
| & | & | \\
\text{(CHOH)}_3 & \text{(CHOH)}_3 & \text{(CHOH)}_3 \\
| & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
\end{array}
$$
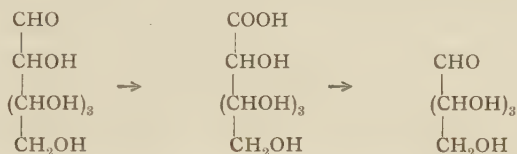
Full details of these reactions must be obtained from larger textbooks and special books on carbohydrates.

Glucose, Grape Sugar, or Dextrose.

Glucose occurs in the seeds, leaves, and other parts of plants, and together with fructose in sweet fruits and honey.   It is present to the extent of about ·1 per cent. in the blood of animals and in other organs of the animal body.   It is formed by the hydrolysis of cane sugar and other polysaccharides which contain it.

Small quantities of glucose are most conveniently prepared from cane sugar as follows : 40 gm. of powdered cane sugar are added to a mixture of 5 c.c. of concentrated hydrochloric acid and 120 c.c. of 90 per cent. alcohol heated to 45-50°.   The mixture is kept at this temperature for 2 hours with occasional stirring and allowed to cool.   Glucose crystallises out on cooling, more rapidly after adding a crystal of anhydrous glucose which helps the crystallisation.   The crystals are filtered off, washed with alcohol, and recrystallised from a mixture of 2 parts of alcohol and 1 of water.

Commercially, glucose is prepared from the starch of potato, maize, etc.   The starch is hydrolysed by heating in copper vessels with dilute sulphuric acid under 3 atmospheres pressure.   The solution is neutralised with chalk, the calcium sulphate filtered off, and the filtrate heated with animal charcoal to decolorise it.   It is evaporated *in vacuo* to a syrup

and allowed to stand.   The glucose crystallises in a cake of small crystals, which are purified by crystallisation from dilute alcohol.

Glucose crystallises from alcohol, or concentrated aqueous solutions at 30°, in needles which are anhydrous.   It crystallises from cold water in the form of plates of the composition $C_6H_{12}O_6 . H_2O$.   It is easily soluble in water, very slightly soluble in absolute alcohol, but more soluble in methyl alcohol.   It is insoluble in ether.   Glucose and other carbohydrates are difficult to prepare free from moisture.   This can only be effected by heating them *in vacuo* at 70-110° in a vessel connected to phosphorus pentoxide.

Fructose, Fruit Sugar, or Laevulose.

Fructose occurs with glucose in fruits, honey, etc.   It is most easily prepared from the polysaccharide, inulin, by boiling it with 5 parts of ·5 per cent. sulphuric acid, or dilute oxalic acid, for 1 hour.   The acid is removed with barium carbonate and the solution is treated with charcoal and evaporated to a syrup.   The syrup is dissolved in alcohol from which fructose slowly crystallises out.

On a large scale, fructose is made from cane sugar.   The solution of cane sugar which has been hydrolysed by dilute acid is neutralised and treated with milk of lime.   An insoluble calcium compound of fructose is formed ; this is filtered off, decomposed with carbon dioxide, and the fructose obtained as above.

Fructose crystallises from alcohol in the form of rhombic crystals ; it crystallises from water in needles of the composition $2C_6H_{12}O_6 . H_2O$. It is soluble in hot absolute alcohol and can thus be separated from other sugars.

Mannose.

Mannose does not occur as such in nature, but is widely distributed as the polysaccharide, mannan.   It can be obtained by the oxidation of mannitol, but is usually prepared by the hydrolysis of the mannan contained in ivory-nut, which is used in making buttons.   The material is hydrolysed by heating it on a water-bath for 6 hours with twice its weight of 6 per cent. hydrochloric acid.   The insoluble matter is removed by filtration and the solution is decolorised by heating with animal charcoal, neutralised, and treated with phenylhydrazine acetate.   Mannose phenyl-hydrazone is formed, from which the sugar is obtained by decomposing it with cold concentrated hydrochloric acid.   Mannose is a hard colourless solid, which deliquesces, is easily soluble in water, slightly soluble in alcohol, and insoluble in ether.

Galactose.

Galactose occurs in combination with glucose in milk-sugar, or lactose, in some gums and seaweeds as the polysaccharide, galactan, and in some glucosides, e.g. xanthorhamnin and saponin of plants, the cerebrosides of the brain and nervous tissue of animals.

It is prepared from lactose by boiling it with 4 times its weight of 2 per cent. sulphuric acid for 6 hours. The solution is concentrated and allowed to crystallise. The crude galactose is recrystallised by dissolving it in four-fifths of its weight of water and adding 2 volumes of 93 per cent. alcohol. Galactose consists of very small hexagonal crystals which melt at 168°.

d-Glucosamine.

The hydrochloride of glucosamine, or aminoglucose, was obtained in 1878 from chitin, the organic constituent of the shells of the lobster. It has since been obtained from the organic material of the shells of other arthropods. It is a constituent of fungus cellulose and has been prepared from various glucoproteins. These conjugated proteins contain glucosamine, or a polysaccharide composed of glucosamine, as their carbohydrate moiety.

d-Glucosamine was synthesised by Fischer and Leuchs from d-arabinose and was shown to have the formula,

$$CH_2OH \cdot \overset{H}{\underset{OH}{C}} \cdot \overset{H}{\underset{\diagdown}{C}} \cdot \overset{OH}{\underset{H \ H_2N}{C}} \cdot \overset{H}{\underset{\diagup}{C}} \cdot CHOH$$
$$O$$
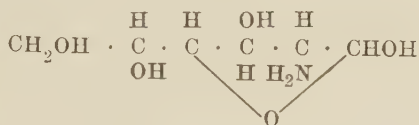
Preparation.

Lobster or crab shells, which have been treated with dilute hydrochloric acid to decompose the calcium carbonate and washed with water, are gently boiled with concentrated hydrochloric acid for 3-4 hours. The solution is evaporated until it crystallises and allowed to cool. The dark brown crystals of glucosamine hydrochloride are filtered off, dissolved in water, and the solution evaporated until it crystallises. Glucosamine may be obtained from the hydrochloride by the action of diethylamine, or sodium methylate.

Properties.

Glucosamine is a very unstable base and is known chiefly in the form of its hydrochloride.

Glucosamine hydrochloride forms large colourless glistening crystals which are soluble in water.

It cannot be converted directly into glucose; by the action of nitrous acid it is converted into chitose, which Fischer and Andreae have shown to be a furfurane derivative of the formula,

$$HO \cdot CH - CH \cdot OH$$
$$CH_2OH \cdot \underset{\diagdown}{CH} \quad \underset{\diagup}{CH} \cdot OH$$
$$O$$
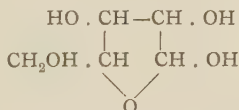
Glucosamine is hence frequently termed chitosamine.

It resembles glucose closely in its properties; it forms a pentacetyl derivative, an oxime and a phenyl-hydrazone and yields an osazone identical with glucosazone.

Its solutions behave like those of glucose as a reducing agent to Fehling's solution, etc.

REACTIONS OF THE MONOSACCHARIDES.

The reactions of the monosaccharides are very similar, the differences between the individual members being only in certain peculiarities. The reactions of glucose may be taken as typical of the reactions of all monosaccharides.

A. GLUCOSE.

(1) **Action of Alkali.**—Glucose is acted upon by sodium hydroxide at 37°. The rotation of the solution diminishes and its acidity increases. It passes over into fructose and mannose. Carbonates have a slighter action and ammonia of the same concentration is almost without action.

Moore's Test.—On boiling a solution of glucose with sodium hydroxide, it turns yellow, then dark brown, and smells of caramel. The smell becomes more distinct on acidifying the solution with dilute sulphuric acid and its colour becomes lighter. Lactic acid and other acids are formed.

(2) **Action of Concentrated Hydrochloric Acid.**—If a solution of glucose be boiled for some time with an equal volume of concentrated hydrochloric acid, the solution becomes brown and "humus" substances, which are black, separate out. The chief product of the action of hydrochloric acid on glucose is levulinic acid, or acetylpropionic acid,

$$CH_3 . CO . CH_2 . CH_2 . COOH \text{ (p. 160)}.$$

(3) **Reduction of Metallic Oxides in Alkaline Solution.**

(*a*) *Silver.*—On adding a solution of glucose to some ammoniacal silver nitrate solution (prepared by adding dilute ammonia to silver nitrate until the precipitate first formed is just redissolved) and warming in the water-bath, a mirror of metallic silver gradually forms.

(*b*) *Copper.*

(i) *Trommer's Test.*—On making a solution of glucose alkaline with sodium hydroxide and adding copper sulphate, drop by drop, shaking after each addition, the solution becomes deep blue. The addition of excess of copper sulphate causes the precipitation of cupric hydrate, i.e. it is no longer dissolved by the glucose solution. The addition of a few small crystals of Rochelle salt will redissolve the precipitate (see Fehling's test). On heating the clear blue solution nearly to boiling, a yellowish-red precipitate of cuprous oxide is formed.

*(ii) *Fehling's Test.*—On adding some glucose solution to equal quantities of Fehling's solution (*a*) $CuSO_4$, (*b*) NaOH + NaK Tart. and heating to boiling, cuprous oxide is precipitated.

It should be noted that ammonium salts modify the reaction; the cuprous oxide is not precipitated, but the blue colour of the solution becomes less intense and may disappear.

*(iii) *Benedict's Test.*—As glucose is destroyed by the action of sodium hydroxide, the reaction is more sensitive if sodium carbonate be employed in its place. If sodium citrate be substituted for Rochelle salt, a permanent solution (Benedict's qualitative reagent) is obtained.

On adding 5 to 10 drops of glucose solution to about 5 c.c. of the reagent and boiling vigorously for 2 or 3 minutes, it becomes turbid with a red, yellow, or green precipitate which fills up the solution. The final colour depends on the amount of glucose. If the amount of glucose be very small, a precipitate is only observed on allowing the solution to cool.

The test is sensitive to ·08 per cent. of glucose.

*(iv) *Barfoed's Test.*—Glucose also reduces cupric hydrate in acetic acid solution. If some glucose solution be added, drop by drop, to some Barfoed's reagent which is kept boiling during the addition, red cuprous oxide is precipitated.

This test is given by glucose and other monosaccharides, but not by lactose and maltose. It may be used for distinguishing between glucose and the disaccharides, but the reagent must be freshly prepared, otherwise maltose and lactose will also reduce it.

(*c*) *Bismuth.*

Boettger's Test.—On boiling some glucose solution with a few crystals of bismuth subnitrate and twice the quantity of sodium carbonate, the bismuth hydrate first formed becomes reduced to metallic bismuth; the precipitate becomes grey, or black, in colour.

Nylander's Test.—If 5 parts of glucose solution be boiled with 1 part of Nylander's reagent for 2-5 minutes, reduction occurs and a black precipitate settles out on cooling.

These reactions are particularly useful for detecting small quantities of glucose in urine. The uric acid and creatinine in urine also reduce Fehling's solution, but not Nylander's solution.

(4) Reduction of Dye-stuffs.

(i) On adding picric acid and caustic soda to a solution of glucose and warming, a blood-red colour is formed due to the formation of picramic acid.

(ii) On warming a dilute solution of sodium sulphindigotate, made alkaline with sodium carbonate, with some glucose solution, the blue colour changes to green, purple-red, and finally yellow. The blue colour returns on cooling and shaking the solution with air.

(iii) If some glucose solution be added to about 5 c.c. of a solution of safranin and the mixture boiled, the opaque red colour changes to light yellow.

(5) **Formation of Osazones.**—The reaction of glucose and other reducing sugars with phenylhydrazine is very characteristic, as it serves for the identification of the different carbohydrates (see below).

FIG. 32.—Glucosazone.

* On adding equal quantities of phenylhydrazine and glacial acetic acid (5 to 10 drops of each), or 1 part of phenylhydrazine hydrochloride and 2 parts of sodium acetate to about 20 c.c. of glucose solution and warming in a boiling water-bath for half to one hour, a yellow crystalline mass of phenylglucosazone is formed. The solution is allowed to cool, the crystals are filtered off and examined under a microscope. They consist of long needles arranged in sheaves as in Fig. 32.

* (6) **Fermentation.**—Glucose is fermented by yeast into alcohol and carbon dioxide. If a little fresh yeast be rubbed up with some glucose solution and a test tube be filled with the mixture and inverted in warm water in a crucible at 25°, it will be seen that after a short time bubbles rise to the top and displace the liquid. In about 24 hours most of the glucose will have disappeared and alcohol can be detected in the liquid.

* (7) **Molisch's Test.**—On adding a drop of α-naphthol solution to about 0·5 c.c. of glucose solution and some water and running about 5 c.c. of concentrated sulphuric acid below it, a purple ring appears at the surfaces of contact, either at once, or after a short time. The two liquids may be mixed, but the mixture must be kept cold by holding under running water. The whole liquid becomes reddish-violet. An examination of the coloured solution with a spectroscope will show an absorption band between D and E, whilst the violet end is totally

absorbed (cf. p. 476).

$$\begin{array}{cc} H.C & C.H \\ || & || \\ CH_2OH.C & C.CHO \\ & \diagdown\diagup \\ & O \end{array}$$

ω-Hydroxymethyl furfural, which gives the pigment with a-naphthol, is formed.
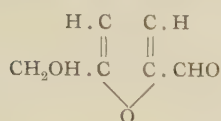
This reaction is the most general one for all carbohydrates. It is most effective for small quantities and is really only useful for detecting carbohydrate in proteins.

(8) **Rotation.**—Glucose in solution is dextrorotatory when examined with a polarimeter, and shows mutarotation—the initial high rotatory power decreases and becomes constant in about 24 hours, or on boiling, or on adding a drop of ammonia.

(9) **Rubner's Test.**—If to about 10 c.c. of 2 per cent. glucose solution, 3 gm. of lead acetate and 2 c.c. of ·880 ammonia be added, and the mixture placed in a boiling water-bath, a pink colour develops in about 30 seconds.

B. FRUCTOSE.

Fructose gives all the reactions given by glucose, but the following differences should be noted :—

(1) **Action of Alkali.**—Fructose is changed to caramel rather more rapidly than glucose.

(2) **Action of Concentrated Hydrochloric Acid.**—On boiling a solution of fructose with concentrated hydrochloric acid, the solution generally becomes red, or red-brown, before it ultimately turns dark brown.

(3) **Reduction of Metallic Oxides in Alkaline Solution.**—Although fructose is a ketose, it nevertheless reduces metallic oxides in alkaline solution. This is due to the terminal—$CO . CH_2OH$ group which is easily oxidisable. Though acetone does not reduce metallic oxides, monohydroxyacetone $CH_3 . CO . CH_2OH$ does, as it contains the above grouping.

(5) **Formation of Osazones.**—Fructose gives a phenylosazone identical with phenylglucosazone, as can be seen when the crystals are examined under the microscope, melting-point, analysis, etc. (see below).

(6) **Fermentation.**—Fructose ferments more rapidly than glucose with yeast.

(8) **Rotation.** — Fructose solutions are laevorotatory. Laevorotatory fructose is known as d-fructose on account of its stereochemical relationship to glucose ; the asymmetry of glucose is the basis of the stereochemical configuration of all carbohydrates.

Special Test. Selivanoff's Test.—On adding a few crystals of resorcinol to a mixture of equal parts of concentrated hydrochloric acid and water and a very small quantity of fructose solution and

heating, the solution becomes red in colour and deposits a brownish-red precipitate, which dissolves in alcohol giving a red solution.

ω-Hydroxymethylfurfural is formed by the action of the acid upon fructose and combines with the resorcinol giving the red pigment.

C. GALACTOSE.

Galactose gives the same reactions as glucose. It differs from glucose in the following particulars :—

(5) **Formation of Osazones.**—Galactose gives a different phenylosazone.

(6) **Fermentation.**—Galactose is fermented by yeast, but much less rapidly than glucose.

(8) **Rotation.**—Galactose has a higher dextrorotatory power than glucose.

D. MANNOSE.

Mannose differs in the following particulars from glucose :—

(5) **Formation of Hydrazone and Osazone.**—Mannose forms a phenylhydrazone which is soluble with difficulty in water. It forms the same phenylosazone as glucose.

(8) **Rotation.**—Mannose is dextrorotatory, but has a different rotatory power.

Formation of Osazones.

The reaction of glucose, mannose, and fructose and other monosaccharides with phenylhydrazine takes place in two stages :—

(1) In the first stage the ordinary reaction of an aldehyde, or a ketone, takes place, giving the hydrazone :—

$$\begin{array}{l} CHO \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + H_2N.NH.C_6H_5 \quad = \quad \begin{array}{l} CH=N.NHC_6H_5 + H_2O \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

Glucose, or mannose.   Glucose, or mannose, Phenylhydrazone.

$$\begin{array}{l} CH_2OH \\ | \\ CO \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + H_2N.NH.C_6H_5 \quad = \quad \begin{array}{l} CH_2OH \\ | \\ C=N.NH.C_6H_5 + H_2O \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

Fructose.   Fructose phenylhydrazone.
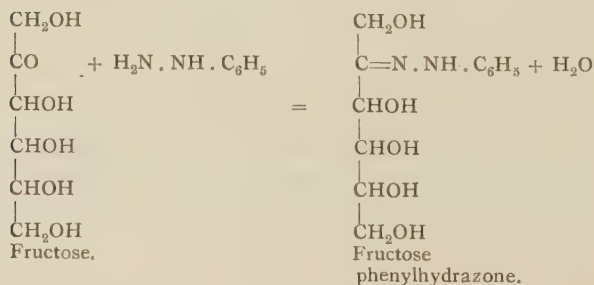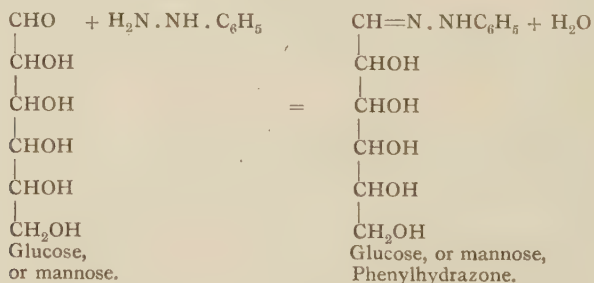
The phenylhydrazones of glucose and fructose are soluble in water, that of mannose is soluble with difficulty, and may crystallise out.

(2) In the second stage, the secondary alcohol grouping on carbon atom 2 in the case of the aldohexoses, and the primary alcohol grouping on carbon atom 1 in the case of fructose is oxidised by the phenylhydrazine to a ketone, or aldehyde, group :—

$$
\begin{array}{l}
CH=N.NH.C_6H_5 \\
| \\
CHOH \\
| \\
CHOH \quad + H_2N.NHC_6H_5 = \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\qquad
\begin{array}{l}
CH=N.NH.C_6H_5 \\
| \\
CO \\
| \\
CHOH \quad + C_6H_5.NH_2 + NH_x \\
| \qquad\qquad \text{Aniline.} \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH \\
\text{Glucosone} \\
\text{phenylhydrazone.}
\end{array}
$$

$$
\begin{array}{l}
CH_2OH \\
| \\
C=N.NH.C_6H_5 \\
| \\
CHOH \quad + H_2N.NH.C_6H_5 = \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\qquad
\begin{array}{l}
CHO \\
| \\
C=N.NH.C_6H_5 \\
| \\
CHOH \qquad + C_6H_5.NH_2 + NH_2 \\
| \qquad\qquad\quad \text{Aniline.} \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH \\
\text{Fructosone} \\
\text{phenylhydrazone.}
\end{array}
$$
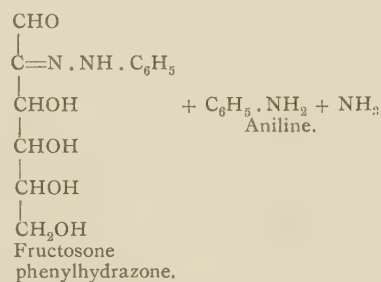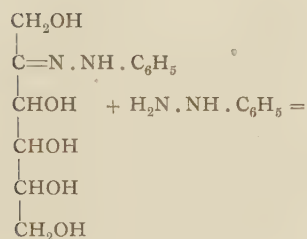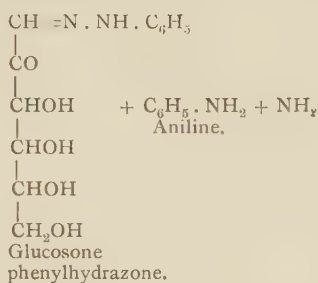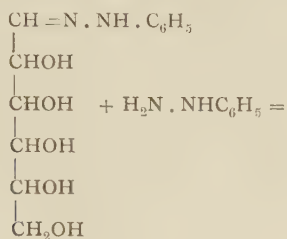
(3) In the third stage, phenylhydrazine reacts with the ketone, or aldehyde, so formed :—

$$
\begin{array}{l}
CH=N.NH.C_6H_5 \\
| \\
CO \qquad + H_2N.NH.C_6H_5 \\
| \\
CHOH \qquad\qquad = \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\qquad
\begin{array}{l}
CH=N.NH.C_6H_5 \\
| \\
C=N.NH.C_6H_5 \\
| \\
CHOH \qquad + H_2O \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH \\
\text{Phenyl glucosazone.}
\end{array}
$$

$$
\begin{array}{l}
CHO \qquad + H_2N.NH.C_6H_5 \\
| \\
C=N.NH.C_6H_5 \\
| \\
CHOH \qquad\qquad = \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\qquad
\begin{array}{l}
CH=N.NH.C_6H_5 \qquad + H_2O \\
| \\
C=N.NH.C_6H_5 \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH \\
\text{Phenyl glucosazone.}
\end{array}
$$
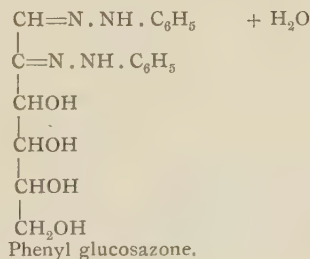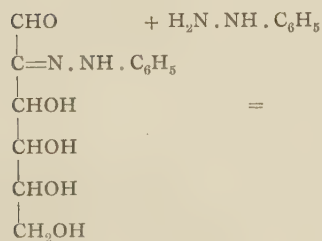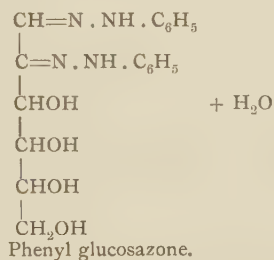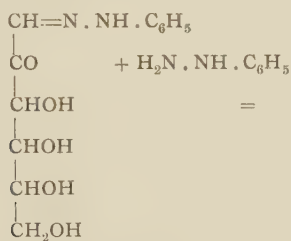
The same phenyl glucosazone is formed from glucose, mannose, and fructose.   The reaction will be seen to occur with carbon atoms 1 and 2 of the carbohydrate.   It is only in respect to the different asymmetry of carbon atom 2, that these three monosaccharides differ.   The asymmetry of carbon atom 2 is lost in the formation of the osazone and hence the identity of the osazones.

Glucosazone is decomposed by boiling with concentrated hydrochloric acid, with the formation of glucosone.   Glucosone is reduced by sodium amalgam to fructose.   Glucose and mannose can thus be changed to fructose :—

$$
\begin{array}{ccccc}
CH = N.NH.C_6H_5 & & CHO & & CH_2OH \\
| & & | & & | \\
C = N.NH.C_6H_5 & & CO & & CO \\
| & & | & & | \\
CHOH & \rightarrow & CHOH & \rightarrow & CHOH \\
| & & | & & | \\
CHOH & & CHOH & & CHOH \\
| & & | & & | \\
CHOH & & CHOH & & CHOH \\
| & & | & & | \\
CH_2OH & & CH_2OH & & CH_2OH \\
\text{Glucosazone.} & & \text{Glucosone.} & & \text{Fructose.}
\end{array}
$$
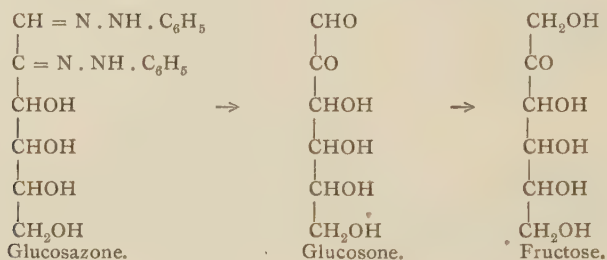
E. PENTOSES.

The pentoses give most of the reactions given by glucose but with the following differences :—

(1) **Action of Alkali.**—Pentoses are turned yellow, or brown, on boiling with caustic alkali.

(2) **Action of Concentrated Hydrochloric Acid.**—The pentoses on boiling with hydrochloric acid yield furfural, which is volatile with steam and may be detected with aniline acetate.

*   If a solution containing pentose,[1] or pentosan, e.g. gum arabic solution, be boiled with about half its volume of hydrochloric acid and if a piece of filter paper moistened with aniline acetate solution (prepared from equal parts of aniline, glacial acetic acid, and water) be held in the vapour escaping from the vessel after most of the hydrochloric acid has been evolved, a bright crimson colour will be formed.

*   (3) **Reduction of Metallic Oxides in Alkaline Solution.**—Pentoses reduce Fehling's solution on warming for some time.

(5) **Formation of Osazones.**—The pentoses form phenylosazones with phenylhydrazine in acetic acid solution.   They differ from phenylglucosazone in melting-point, analysis, etc.

---

[1] A solution containing arabinose may be readily prepared by boiling 5 gm. of gum arabic in 100 c.c. water with 10 c.c. of concentrated hydrochloric acid for 5 minutes and then neutralising with alkali.

(6) **Fermentation.**—Pentoses are not fermented by yeast.

(8) **Rotation.**—Pentoses are dextrorotatory, or inactive.

Special Tests.

* (1) *Phloroglucinol Reaction.*—On adding an equal volume of concentrated hydrochloric acid and a small quantity of phloroglucinol to a solution of a pentose and heating the mixture in a boiling water-bath, it gradually becomes cherry-red in colour and turbid and a precipitate is formed. The precipitate is dissolved by amyl alcohol, if the cold solution be shaken up with this solvent; the amyl alcohol solution will show, on examination with a spectroscope, an absorption band between D and E.

The formation of a precipitate is not itself sufficient evidence for the presence of a pentose, since a precipitate may be formed on heating other substances with acid and phloroglucinol.

* (2) *Orcinol Reaction* (Tollens).—If a mixture of equal parts of concentrated hydrochloric acid and pentose, or pentosan solution, be heated with a little orcinol, the solution becomes red, then violet, finally blue or blue-green with the separation of a precipitate. The appearance of the green colour may be hastened by adding a drop of ferric chloride to a *portion* of the solution. The *remainder* of the solution on being shaken with amyl alcohol imparts a bluish-red colour to the amyl alcohol, which gradually becomes green. The solution, on examination with a spectroscope, shows an absorption band between C and D but near D.

Bial's Modification.—On adding Bial's reagent, drop by drop, to about 5 c.c. of a boiling solution of a pentose, a bright green colour is produced. This is soluble in amyl alcohol, as above.

F. GLYCURONIC ACID.

Glycuronic acid resembles the pentoses in its reactions.

A solution of glycuronic acid may be prepared by boiling a small quantity of Indian yellow with dilute hydrochloric acid, cooling, filtering off the euxanthone and neutralising the solution.

(1) It reduces Fehling's solution.

(2) It gives the phloroglucinol and orcinol reactions.

(3) It does not ferment.

(4) The free acid is dextrorotatory, but when combined with euxanthone, or other compounds, it is laevorotatory.

THE DISACCHARIDES.

The disaccharides consist of two units of monosaccharide combined together with loss of water. Theoretically, any two monosaccharides can be thus combined, but actually only a few disaccharides are known.

Most of these are natural compounds, but some have been obtained by synthesis. Their composition is shown by hydrolysis—by acids, or enzymes—when they are converted into their constituents, e.g. in the case of a bihexose :—

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$
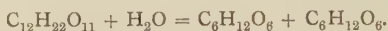
The natural disaccharides are of two kinds, those which reduce Fehling's solution and those which do not, and they are usually classified accordingly :—

Non-Reducing.		*Reducing.*			
Sucrose or Saccharose, or Cane Sugar	⌠Glucose ⎨ ⎩Fructose	Lactose or Milk Sugar	⌠Glucose ⎨ ⎩Galactose	Isolactose	⌠Glucose ⎨ ⎩Galactose
		Melibiose	⌠Glucose ⎨ ⎩Galactose		
Trehalose	⌠Glucose ⎨ ⎩Glucose	Maltose	⌠Glucose ⎨ ⎩Glucose	Isomaltose	⌠Glucose ⎨ ⎩Glucose
		Gentiobiose	⌠Glucose ⎨ ⎩Glucose		
		Cellobiose	⌠Glucose ⎨ ⎩Glucose		
		Turanose	⌠Glucose ⎨ ⎩Fructose		
		Vicianose	⌠Glucose ⎨ ⎩Arabinose	Galacto-arabinose	⌠Galactose ⎨ ⎩Arabinose
		Gluco-xylose	⌠Glucose ⎨ ⎩Xylose		

It is not yet definitely known how the two units are combined together. In the non-reducing members the two functional aldehyde, or ketone, groupings will be combined; in the reducing members the aldehyde, or ketone, grouping of the one unit will be united to one of the hydroxyl groups of the other unit. The following are the probable formulæ for

Cane Sugar

$$CH_2OH \cdot C \cdot (CHOH)_2 \cdot CH \cdot CH_2OH \qquad \text{fructose residue}$$

$$CH \cdot (CHOH)_2 \cdot CH \cdot CHOH \cdot CH_2OH \qquad \text{glucose residue}$$

Trehalose

$$CH_2OH \cdot CHOH \cdot CH \cdot (CHOH)_2 \cdot CH$$
$$CH_2OH \cdot CHOH \cdot CH \cdot (CHOH)_2 \cdot CH$$

Maltose

$$CH_2OH \cdot CHOH \cdot CH \cdot (CHOH)_2 \cdot CH-O \cdot CH \cdot CHOH \cdot CH \cdot (CHOH)_2 CHOH$$
$$\underset{O}{\smile} \qquad\qquad \underset{O}{\smile}$$

Lactose

$$CH_2OH \cdot CHOH \cdot \overset{\frown}{CH} \cdot (CHOH)_2 \overset{O}{\cdot} CH-O \cdot CH_3 \quad CHOH \cdot \overset{\frown}{CH} \cdot (CHOH)_2 CHOH$$

or

$$CH_2OH \cdot CHOH \cdot \overset{\frown}{CH} \cdot (CHOH)_2 \cdot CH-O \cdot \underset{}{\overset{CH_2OH}{\underset{|}{CH}}} \cdot \overset{\frown}{CH} \cdot (CHOH)_2 \cdot CHOH$$
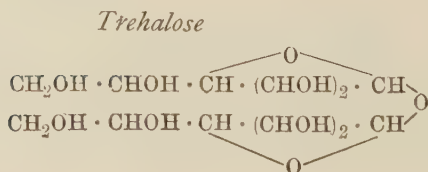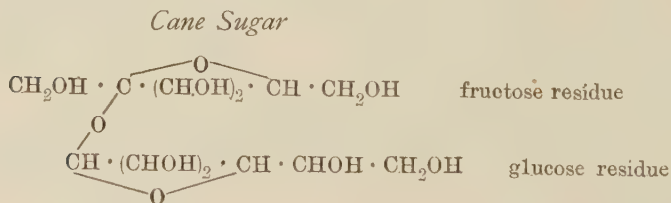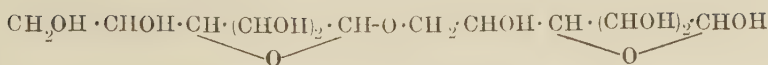
galactose residue   glucose residue
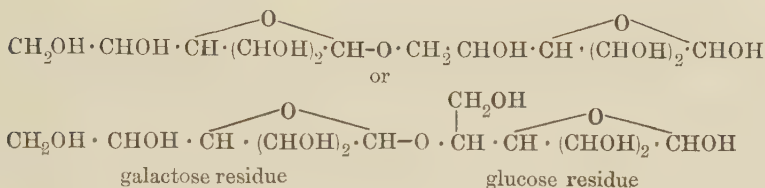
The large number of possible disaccharides may be due partly to the possibility of combination with the several hydroxyl groups, and partly to the possibility of the combination of *a*- or *β*- forms of the constituents, thus

$$a—a, \quad \beta—a,$$
$$a—\beta, \quad \beta—\beta.$$
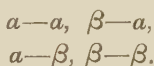
Cane sugar, lactose, and maltose are the chief and commonest disaccharides.

CANE SUGAR.

Cane sugar is very widely distributed in the vegetable kingdom: 20 per cent. is present in the juice of the sugar cane, 10-20 per cent. in beetroot; smaller quantities are present in the maple and birch. Sweet fruits contain cane sugar together with glucose and fructose, which are probably derived from it by hydrolysis; 5-12 per cent. of cane sugar is present in bananas, apricots, strawberries and pineapple. The mixture of glucose and fructose in honey is probably the result of the hydrolysis of cane sugar of the flowers by the formic acid secreted by the bees.

Preparation.

Cane sugar is prepared mainly from the cane and beet, though other plants, e.g. maple, palm, are used as sources of cane sugar. The manufacture in all cases is very similar. The juice of the cane, prepared by crushing the cane and pressing out, or the aqueous extract of beet, prepared by diffusion in a series of vessels, is treated with milk of lime to neutralise acids and boiled to precipitate proteins. The solution is treated with carbon dioxide to remove the last traces of calcium and with sulphur dioxide to decolorise it. It is again boiled and filtered and evaporated *in vacuo* until it crystallises. The residue, termed molasses, which does not readily crystallise yields more cane sugar on treatment of the boiling solution with lime, or strontia, by which means an insoluble calcium, or strontium, saccharate is formed. The solid is separated

18 *

and decomposed with carbon dioxide and the solution yields cane sugar on evaporation.   Cane sugar molasses are most frequently fermented and converted into rum.

Properties.

Cane sugar in contrast to other sugars crystallises extremely readily and forms colourless monoclinic crystals easily soluble in water and only slightly soluble in alcohol.   A saturated solution contains 66 per cent. of cane sugar.   It melts on heating to about 160° to a glassy mass termed barley sugar which gradually crystallises again.   If it be further heated to about 200°, it is changed into a brown substance, caramel, which does not crystallise.

Cane sugar forms esters with eight hydroxyl groups.

Cane sugar forms insoluble compounds with lime, strontia, lead hydroxide, etc., more easily than glucose; this property as mentioned above is made use of in its commercial preparation.

Invert Sugar.—On hydrolysis, cane sugar is converted into equal parts of glucose and fructose :—

$$C_{12}H_{22}O_{11} = C_6H_{12}O_6 + C_6H_{12}O_6$$
$$\text{Glucose.} \qquad \text{Fructose.}$$
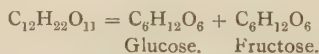
This mixture reduces Fehling's solution and shows laevorotation due to the laevorotation of fructose being greater than the dextrorotation, of glucose.   Owing to the change of rotation, or inversion, the mixture of glucose and fructose obtained from cane sugar is generally spoken of as invert sugar.

Reactions of Cane Sugar.

Cane sugar differs considerably from glucose in many of its reactions.

* (1) *Action of Alkali.*—Cane sugar, since it contains no aldehyde, or ketone, group, is acted upon by alkali and does not give Moore's test.

* (2) *Action of Hydrochloric Acid.*—Cane sugar is easily hydrolysed by boiling with dilute hydrochloric acid into glucose and fructose. Concentrated hydrochloric acid has the same action upon it as upon fructose (and glucose).   The solution turns red, and then brown.

* (3) *Reduction of Metallic Oxides in Alkaline Solution.*—Cane sugar does not reduce Fehling's solution, etc.   Cane sugar, after hydrolysis by boiling with dilute acid and neutralisation of the acid with sodium hydroxide, reduces Fehling's solution, etc.

* (5) *Formation of Osazones.*—Cane sugar does not form a phenyl-

osazone. After hydrolysis by acids into glucose and fructose, phenyl-glucosazone is formed.

(6) *Fermentation.*—Cane sugar is fermented by yeast, but before fermentation into carbon dioxide and alcohol it is converted by hydrolysis by the enzyme, invertase, in the yeast into glucose and fructose.

(7) *Molisch's Test.*—Cane sugar gives Molisch's reaction.

(8) *Rotation.*—Cane sugar is dextrorotatory. After hydrolysis by acids, the mixture of glucose and fructose in equal parts shows laevo-rotation.

(9) *Selivanoff's Test.*—Cane sugar gives Selivanoff's reaction, since it contains fructose.

LACTOSE.

Lactose, or milk sugar, occurs in the milk of all animals, but has not been found in plants; about 4 per cent. is present in cow's milk, from 6-8 per cent. in human milk.

Lactose is prepared and manufactured from whey obtained in the preparation of cheese. The whey is evaporated and the lactose crystallises out; it is purified by recrystallisation from water.

Lactose forms a white crystalline powder, soluble in water, but insoluble in alcohol. In taste it is less sweet than glucose, or cane sugar.

Lactose forms esters with eight hydroxyl groups.

On oxidation of lactose with nitric acid, a mixture of mucic and saccharic acids is formed.

Reactions of Lactose.

Lactose resembles glucose in its reactions.

(1) *Action of Alkali.*—Lactose gives Moore's test.

(2) *Action of Hydrochloric Acid.*—Lactose is hydrolysed by boiling with dilute acid into a mixture of glucose and galactose. Strong acid has the same effect as upon glucose.

(3) *Reduction of Metallic Hydroxides in Alkaline Solution.*—Lactose reduces Fehling's solution. The reducing power of lactose is less than that of glucose. (See under estimation.) After hydrolysis by acids the mixture of glucose and galactose has a greater reducing power than lactose.

Lactose does not reduce Barfoed's reagent.

(5) *Formation of Osazone.*—Lactose forms an osazone with phenyl-hydrazine in acetic acid solution in the same way as glucose. Lac-tosazone is soluble in boiling water; the compound separates as the

solution cools. Its crystalline form (Fig. 33) is different from that of glucose and it is thus most easily distinguished from glucosazone and also maltosazone.

FIG. 33.—Lactosazone.

* (6) *Fermentation.* — Lactose is not fermented by yeast.

* (7) *Molisch's Test.* — Lactose gives Molisch's reaction.

* (8) *Rotation.* — Lactose is dextrorotatory. After hydrolysis by acids into a mixture of glucose and galactose, the rotatory power of the solution is greater than before.

(9) *Rubner's Test.* — A 2 per cent. solution of lactose, treated with lead acetate and ammonia, does not turn pink rapidly like glucose, but may do so on prolonged heating.

MALTOSE.

Maltose is found in plants and is formed in considerable quantities from starch during the germination of barley and other cereals. The polysaccharide is hydrolysed by the enzyme, diastase, in the grain into a mixture of maltose and dextrin :—

$$(C_6H_{10}O_5)n + H_2O = C_{12}H_{22}O_{11} + (C_6H_{10}O_5)n - 2.$$

Maltose is also formed by the careful hydrolysis of starch by acids, and also from glycogen by the action of diastase.

Diastase prepared from barley (30 gm.) (see p. 406) is added to 30 gm. of starch, or soluble starch, in 3000 c.c. of water. The mixture is kept at 50° for 3 hours and then for 12 hours at room temperature. 60 per cent. of maltose is formed. The solution is filtered, evaporated to a thin syrup, and poured into 95 per cent. alcohol. The precipitate of dextrin is removed and the alcohol distilled from the solution. Maltose separates out on standing. It is purified by dissolving in a little water, pouring into boiling alcohol, filtering, removing the alcohol and allowing to crystallise.[1]

[1] Baker and Day, Brit. Assoc. Report, 1908, Sect. B., 671.

Maltose is readily soluble in water from which it crystallises in white needles of the composition $C_{12}H_{22}O_{11}$. H_2O.   It forms esters with eight hydroxyl groups.

Reactions of Maltose.

Maltose resembles glucose in its reactions more closely than lactose.

* (1) *Action of Alkali.*—Maltose gives Moore's test.

* (2) *Action of Hydrochloric Acid.*—Maltose is hydrolysed by boiling with dilute hydrochloric acid into two molecules of glucose. Concentrated hydrochloric acid has the same action upon it as upon glucose.

* (3) *Reduction of Metallic Hydroxides in Alkaline Solution.*—Maltose reduces Fehling's solution, etc., but its reducing power is less than that of glucose.   After hydrolysis by acids, the reducing power of the solution is greater than before hydrolysis.

Maltose does not reduce Barfoed's reagent.

* (5) *Formation of Osazone.*—Maltose behaves like lactose in forming an osazone with phenyl-hydrazine in acetic acid solution; it is soluble in boiling water and crystal-lises out as the hot solu-tion cools.   In its appear-ance maltosazone is differ-ent from glucosazone and lactosazone (Fig.   34); also in its melting-point, etc.

(6)   *Fermentation.* — Maltose is fermented by yeast, being converted by the enzyme, maltase, in the   yeast   into   glucose, which yields alcohol and carbon dioxide.

FIG. 34.—Maltosazone.

(7) *Molisch's Test.*—Maltose gives Molisch's reaction.

(8) *Rotation.*—Maltose has a high dextrorotation.   The rotatory power of a solution of maltose diminishes when the maltose is hydro-lysed by acid.

TRISACCHARIDES AND TETRASACCHARIDES.

The number of known compounds in this group is small. They are

Mannotriose,

glucose—galactose—galactose.

Rhamninose,

galactose—rhamnose—rhamnose.

Raffinose,

fructose—glucose—galactose.

Gentianose,

fructose—glucose—glucose.

Melicitose,

glucose—fructose—glucose?

Stachyose,

fructose—glucose—galactose—galactose.

Further details of these carbohydrates are given in Armstrong's monograph, "The Simple Carbohydrates and Glucosides."

THE POLYSACCHARIDES.

The polysaccharides are substances of high molecular weight. The size of their molecule is unknown, but it is composed of a large number of monosaccharide units. Their empirical formula is usually represented by $(C_6H_{10}O_5)_n$, but many polysaccharides contain pentose units as well as hexose units and may consist entirely of pentose units $(C_5H_8O_4)_n$. They may be classified into the following groups :—

Hexosans.—Glucosans :—Starch, dextrin, glycogen, cellulose.

Fructosans :—Inulin.

Mannans.

Galactans.

Pentosans.—Gums, pectins.

Hexosan-Pentosans.—Lignocellulose, hemicellulose.

STARCH.

Starch is present in various parts of plants and has been found in green leaves, fruits, seeds, tubers, etc. The amount of starch present in the seeds of cereals varies from 50-70 per cent. of the dry weight; potatoes contain from 15-30 per cent. It occurs in definite granules—starch grains—which are made up of concentric layers around a hilum. When examined under a microscope, these granules are seen to be of different forms. The source of starch grains can thus be ascertained from their microscopic structure.

Preparation.

Starch is prepared from wheat, rice, maize, potatoes, etc., by mechanical processes. The material is disintegrated by crushing, washed with water and passed through sieves. The starch in suspension passes through and is allowed to settle. The water is drained off and the starch grains are dried.

Starch may be purified by making a 1 per cent. suspension in water, freezing and allowing to melt. The starch is left as a residue, whilst the liquid contains the impurities. The operation is repeated four or five times.

* *Properties.*

Starch grains form a white powder which is insoluble in cold water. If boiled with water, the granules swell and burst, forming an opalescent solution, termed starch paste. Such a solution is most conveniently made by rubbing starch grains into a cream with water and pouring the cream into boiling water and boiling for some minutes. The paste so formed varies in consistency with the amount of starch. Dilute solutions from 1-4 per cent. are limpid, but stronger solutions set into opaque white jellies.

Soluble Starch.

Starch grains consist of at least two substances. The French workers, Maquenne and Roux, term them amylocellulose or amylose, the chief constituent, and amylopectin. Amylose (granulose of previous workers) is partially soluble in boiling water, but completely soluble in boiling water under pressure. On cooling, the insoluble portion is again obtained by "reversion". The one seems to be a polymer of the other. Amylopectin is a gum-like substance which swells up without dissolving in water. The gelatinisation of starch paste is said to be due to the amylopectin.

Fernbach states that soluble starch can be obtained from potato starch by pouring a 1-2 per cent. suspension into a large excess of acetone and shaking vigorously. A flocculent precipitate is formed, which, if filtered off, ground up with acetone in a mortar and dried *in vacuo*, dissolves in cold water.

Starch grains treated with dilute hydrochloric acid of about 10 per cent. for 24 hours do not lose their external appearance, but they become soluble in hot water without forming a paste. Alcohol precipitates from the solution a white powder, soluble in water, termed soluble starch (Brown and Morris).

Soluble starch is most readily prepared by allowing 500 gm. of starch to stand in contact with 1000 c.c. of dilute hydrochloric acid of sp. gr. 1·037 for 7 days, stirring the mixture daily, pouring off the acid, washing the residue free from acid with water by decantation, the last portions containing a trace of ammonia, and drying it by exposure to the air. The dry product, ground up in a mortar and rubbed through a fine hair sieve, is soluble in warm water, giving a clear solution (Lintner).

Another method of preparing soluble starch is to treat 400 gm. of potato

starch with 2300 c.c. of water and 80 c.c. of N HCl in a flask in boiling water for 1·5 hours. .The solution is cooled to 50°, made ammoniacal, and 800 c.c. of alcohol added. The solution is strained through muslin, and whilst warm, poured into 4000 c.c. of alcohol. After 48 hours the precipitate is filtered off, washed with alcohol and spread out to dry.

Reactions of Starch.

The following reactions are given by starch paste, or a solution of soluble starch.

* (1) *Action of Alcohol.*

Starch is precipitated completely by adding an equal volume of alcohol.

* (2) *Action of Iodine.*

If a few drops of iodine solution be added to a starch solution, a dark blue colour appears. On heating the solution, the colour disappears, but appears again on cooling.

The blue colour is discharged on adding 1-2 drops of caustic soda. The colour reappears on neutralising with dilute hydrochloric acid.

* (3) *Basic Lead Acetate.*

On adding basic lead acetate to starch solutions, the starch is precipitated.

* (4) *Ammonium Sulphate.*

Starch is precipitated from solution by adding an equal volume of saturated ammonium sulphate solution, i.e: by half saturation with this salt.

* (5) *Fehling's Solution.*

Starch solutions do not reduce Fehling's solution.

* (6) *Hydrolysis.*

Starch is easily hydrolysed into glucose by boiling its solution with dilute sulphuric acid for a few minutes. The presence of glucose can be shown by neutralising with soda and testing with Fehling's solution.

(7) *Rotation.*

Solutions of soluble starch have a high dextrorotatory power.

DEXTRINS.

Dextrins are ·glucosans which are intermediate in complexity between starch and maltose. They have been found in plants, but are usually obtained by the hydrolysis of starch by the diastase in malt extract.

The existence of a large number of dextrins has been supposed

but only two can be easily distinguished—erythrodextrin, which gives a reddish-brown colour with iodine, and achroodextrin, which gives no colour with iodine. Erythrodextrin is probably a mixture of achroodextrin with a small amount of starch (Ost).

Baker [1] has described a dextrin, termed a-amylodextrin, which results from the action of the diastase of ungerminated barley upon starch paste, or soluble starch. It gives a blue colour with iodine.

Preparation.

The dextrins are prepared by the action of malt extract at 55°, or by the diastase of ungerminated barley at 45-50°, upon starch paste, or soluble starch. The starch is converted by the malt diastase in 2-3 hours into a mixture of 80 per cent. maltose and 20 per cent. dextrin, by the barley diastase into a mixture of 60-65 per cent. of maltose and 35-40 per cent. of dextrin. In the former case the reaction can be followed by testing portions of the solution at intervals with iodine ; the blue coloration disappears, passing through a stage at which a reddish-brown colour is observed.

The prolonged action of malt extract slowly converts the dextrin into maltose. Maquenne and Roux consider that the maltose is derived from amylose and the dextrin from amylopectin.

The solution containing the products of hydrolysis is concentrated and the dextrin is precipitated by pouring it into alcohol. The precipitate is dissolved in water and reprecipitated with alcohol.

Commercial dextrin is prepared by heating starch at 180-200°, until it has a pale brown colour. If the starch be previously treated with acid, it is heated at a lower temperature.

* *Properties.*

Commercial dextrin, obtained by heating starch, is a yellow-brown powder.

The dextrin obtained by the hydrolysis of starch by malt extract is a white powder resembling starch. It is composed chiefly of achroodextrin and is soluble in water, giving a sticky solution. The solution has a faint sweet taste and a peculiar smell.

The dextrin obtained by the action of barley diastase is similar in appearance.

Reactions.

* (1) *Action of A'cohol.*

Dextrin is insoluble in alcohol and is precipitated from its solution in water by excess of alcohol.

[1] J. Chem. Soc., 1902, **81**, 1177.

* (2) *Action of Iodine.*

Commercial dextrin generally gives a reddish-brown coloration on treating with 1-2 drops of iodine solution; this is due to the presence of erythrodextrin. The coloration disappears on heating and reappears on cooling.

The dextrin produced by malt diastase gives no colour with iodine. That produced by barley diastase gives a blue colour.

* (3) *Basic Lead Acetate.*

Dextrin is not precipitated from solution by basic lead acetate.

(4) *Ammonium Sulphate.*

Dextrin is not precipitated by half saturation with ammonium sulphate.

* (5) *Fehling's Solution.*

The dextrins reduce Fehling's solution slightly.

* (6) *Reduction of Dye-stuffs.*

Solutions of dextrin produce a red colour on warming with picric acid and sodium hydroxide.

* (7) *Hydrolysis.*

Dextrin is easily hydrolysed into glucose by boiling its solution with dilute sulphuric acid for a few minutes. The presence of glucose is shown by neutralising the solution with soda and testing with Fehling's solution.

* (8) *Action of Alkali.*

On warming a solution of dextrin with alkali, it becomes yellow, or yellow-brown, in colour.

(9) *Fermentation.*

Dextrin is not fermented by yeast.

(10) *Rotation.*

Dextrin has a high dextrorotatory power.

GLYCOGEN.

Glycogen is present as reserve food material in the organs of animals, but is also found in plants. In plants, it is present in largest amount in yeast, as much as 30 per cent. of the dry weight having been recorded. In animals, glycogen exists in greatest amount in the liver, usually from 1-4 per cent., but 12-16 per cent. have been obtained and 20 per cent. from frog's liver. Glycogen is found in other organs of animals, especially muscle. Heart muscle always seems to contain a quantity of glycogen. Oysters and other molluscs contain considerable quantities of glycogen.

*   *Preparation from Liver.*

In order to obtain as large an amount of glycogen as possible, the animal should be fed on a diet containing carbohydrate. The livers of rabbits contain a considerable quantity, if they have been fed on carrots 5 or 6 hours previously. The animal is killed by bleeding, the liver removed and washed out with saline solution. It is broken up into small pieces and thrown into boiling water acidified with acetic acid. The proteins of the liver are thus coagulated and the enzyme, which converts glycogen into glucose, is destroyed. The pieces of liver are ground up finely in a mortar and extracted with boiling water. The extracts are combined (the remainder of the proteins precipitated by adding an equal volume of 10 per cent. trichloracetic acid), and the opalescent solution precipitated by adding an equal volume of alcohol. The precipitate is redissolved in water and reprecipitated with alcohol. The precipitate is dried by treating with alcohol several times, then with ether and placing in a desiccator over sulphuric acid.

Pfluger's method of preparing glycogen yields a purer preparation :—

The finely broken up liver is stirred up with water and 60 per cent. potassium hydroxide so that it contains 15 per cent. KOH and heated for 2 hours on the water-bath. The solution is filtered and mixed with an equal volume of alcohol. The glycogen is precipitated and washed with a mixture of 1 part of 15 per cent. KOH and 2 parts of alcohol. It may be redissolved and reprecipitated by alcohol. It is then washed with alcohol and ether and dried.

*   *Properties.*

*   Pure glycogen is a white amorphous powder, soluble in cold water, forming an opalescent solution, which is very characteristic.

*   *Reactions.*

*   (1) *Action of Alcohol.*

Glycogen is precipitated from solution by adding an equal volume of alcohol. The precipitation does not occur, if the solution does not contain some salts ; a small quantity, ·05 gm. of sodium chloride, is required to precipitate a 1 per cent. solution of glycogen with two volumes of absolute alcohol.

*   (2) *Action of Iodine.*

Solutions of glycogen give a reddish-brown colour on treatment with 1 to 2 drops of iodine solution. The coloration disappears on heating and reappears on cooling.

*   (3) *Basic Lead Acetate.*

Solutions of glycogen are precipitated by basic lead acetate.

*   (4) *Ammonium Sulphate.*

Solutions of glycogen are not precipitated by half saturation with ammonium sulphate, but the glycogen is precipitated by complete saturation of the solution with ammonium sulphate crystals.

*   (5) *Fehling's Solution.*

Glycogen does not reduce Fehling's solution.

*   (6) *Hydrolysis.*

Glycogen is converted into glucose by hydrolysis.   Solutions of glycogen, boiled with dilute acid and neutralised, reduce Fehling's solution.

*   (7) *Action of Alkali.*

Glycogen is not acted upon by alkali.

(8) *Fermentation.*

Glycogen is not fermented by yeast.

(9) *Rotation.*

Glycogen has a high dextrorotatory power.

CELLULOSE.

The cell walls of plants consist primarily of the substance termed cellulose, but, during growth, the plant forms other substances which are encrusted in the cellulose, so that the material of the cell wall consists of a mixture (or compound) of cellulose and other substances.   The materials which are encrusted in the cellulose are lignin,[1] or lignone, forming lignocellulose in wood, straw, etc., pectins and gummy substances forming pectocellulose, fatty substances forming adipocellulose.   The materials which contain most cellulose, are the fibres of the cotton plant, hemp and flax.   Pure cellulose is generally made from these products.   Wood contains 50-60 per cent. of cellulose; straw contains a similar amount, but silica is also present.

Cellulose is also found in the animal kingdom; the tunicin in the cell walls of tunicates is said to be identical with cellulose.

Preparation of Cellulose from Cotton.

Since cellulose is very resistant to most chemicals, pure cellulose is prepared from cotton fibre by the following treatment :—

The fibre is boiled with 1-2 per cent. caustic potash and washed with water.   Pectins are thus removed.   The fibre is treated with bromine, or chlorine, at the ordinary temperature.   The lignin, or lignone, is destroyed and dissolves.   The fibre is then treated with sodium sulphite, carbonate or hydrate.   The residue is washed with water and dried.

Preparation of Paper Cellulose.

Linen rags, or cotton waste, are cleaned, cut up and boiled under pressure, firstly with dilute sodium carbonate and secondly dilute caustic soda, so as to disintegrate them.   The material is bleached with chlorine, washed free from the halogen, treated with resin, soap and alum, and spread out in thin layers to dry.   The fibres thus become felted together.   Wood is disintegrated and the lignin dissolved out by treatment with calcium bisulphite.   The residue is treated as above with soap, etc.

Paper of an inferior quality is made from wood which has not been treated; it gives the reactions for pentose, if a solution of aniline acetate be

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[1] Lignin appears to contain aromatic substances and pentosans.

poured upon it, or if it be treated with a 1-2 per cent. solution of phloro-glucinol in alcohol and dilute hydrochloric acid.   On exposure to light, such paper becomes yellow.

Properties.

Pure cellulose is a white substance, which is hygroscopic and absorbs about 10 per cent. of water.   The water is removed by heating it to 100°.

Solubility.

It is insoluble in water and all ordinary solvents, but it is decomposed by heating with water under pressure.

It becomes gelatinous in a solution of zinc chloride and finally dissolves. On stirring 1 part of cellulose with 6 parts of zinc chloride in 10 parts of water at 60°, it gelatinises after some time; on raising the temperature by placing it in a boiling water-bath, the cellulose gradually dissolves.

Cellulose dissolves rapidly in a cold solution of zinc chloride in twice its weight of hydrochloric acid.

Cellulose dissolves in a solution of ammoniacal cupric oxide (Schweitzer's reagent).   On adding acid, it is precipitated.

One variety of artificial silk is prepared by dissolving mercerised cotton in Schweitzer's reagent, and running it in a thin stream into dilute sulphuric acid.   A thread of cellulose is thus precipitated.

Reactions.

(1) Action of Alkali.

Dilute solutions (1-2 per cent.) of caustic soda even at 100° have no action upon cellulose.   More concentrated solutions (10 per cent.) cause the fibres to swell and become cylindrical, and destroy the central canal.   The appearance becomes glossy.   This property was used by Mercer for treating cotton to make it appear like silk.

Cellulose treated with 15 per cent. alkali reacts with carbon disulphide, forming a thiocarbonate.   This substance decomposes in the air giving carbon disulphide and cellulose.   The solution, if forced through fine openings and allowed to come into the air, forms continuous threads of artificial silk.

(2) Action of Acids.

Dilute sulphuric acid converts cellulose into hydrocellulose.

Dilute nitric acid (sp. gr. 1·25) at 80° converts cellulose into oxycellulose, which reduces Fehling's solution.

Concentrated sulphuric acid dissolves cellulose.   On diluting the solution, a gelatinous compound is precipitated.   This substance is called amyloid, as it gives a blue colour with iodine, like starch.   Parchment paper is made by treating paper with 2 parts of sulphuric acid and 1 part of water and then washing the acid away with water.

(3) Formation of Esters.

(a) Nitric acid.

Concentrated nitric acid, or a mixture of this acid with sulphuric acid, converts cellulose into nitric acid esters.

Collodion is a mixture of the tri- and tetra-nitrates dissolved in a mixture of equal parts of alcohol and ether.

Celluloid is a mixture of the tri- and tetra-nitrates with camphor.

Gun cotton, or pyroxylin, is cellulose hexanitrate and is prepared by treating cotton waste (freed from fats by treating with alkali) with a mixture of 1 part nitric acid and 3 parts sulphuric acid. The product, which has still the original appearance, is washed with water, moulded and dried. It is converted into smokeless powder by dissolving in acetone, or ethyl acetate, and evaporating the solution. When mixed with nitroglycerine and other substances it forms blasting gelatin, cordite, etc.

Artificial india-rubber is a product prepared by mixing together tri- and tetra-nitrocellulose with castor oil. The inflammability of this material is eliminated by treating it with alkali.

(b) Acetic acid.

Cellulose acetates are obtained on treating cellulose with glacial acetic acid and acetic anhydride in the presence of concentrated sulphuric acid. These compounds are insoluble in water, but soluble in organic solvents. A solution of tetra-acetyl cellulose in acetone on evaporation yields artificial gutta-percha.

A white precipitate is formed when a solution of cellulose acetate in glacial acetic acid is poured into alcohol. This solid does not melt, but burns without leaving an ash. It forms "solid spirit".

It is also used to make artificial silk.

(4) *Hydrolysis.*

Cellulose is dissolved by concentrated sulphuric acid, which hydrolyses it to glucose.

Inulin.

Inulin occurs in the sap of a number of plants and is most abundant in the tubers of the dahlia (10-12 per cent.) and artichoke.

Inulin is prepared from dahlia tubers by crushing and pressing out the juice; the residue yields more inulin, if boiled up with water and chalk. The two solutions are combined, boiled with chalk to neutralise acids, filtered and treated with lead acetate as long as a precipitate is formed. The filtered solution is treated with hydrogen sulphide, filtered from lead sulphide and evaporated to half its volume. An equal volume of alcohol is added and the precipitate of inulin filtered off after 1-2 days. It may be purified by dissolving in water, warming the solution with animal charcoal, filtering and reprecipitating with alcohol. The precipitate is washed with alcohol and ether and dried in a desiccator over sulphuric acid.

Inulin forms a white powder with a sphærocrystalline appearance. It has no taste. It swells up and dissolves in hot water, giving a clear solution.

Reactions.—

(1) *Action of Alcohol.—*Inulin is insoluble in alcohol and is precipitated from solution by adding an equal volume of alcohol.

(2) *Action of Iodine.—*Solutions of inulin give a brownish coloration with iodine. The iodine solution used must be very weak and it is advisable to carry out a control test, i.e. adding the same amount of iodine to an equal volume of water.

(3) *Basic Lead Acetate.—*Inulin solutions are precipitated by basic lead acetate.

(4) *Fehling's Solution.—*Inulin does not reduce Fehling's solution.

(5) *Hydrolysis.—*Inulin is very easily hydrolysed by mineral acids and converted into fructose. The hydrolysed solution, after neutralisation, gives the reactions for fructose.

(6) *Rotation.—*Inulin has lævorotation.

Mannans. Galactans. Hemicellulose, etc.

Polysaccharides, different from those previously described, occur in the seeds of numerous plants. They have resemblances to cellulose, but differ from cellulose in dissolving in dilute alkali, in being hydrolysed by dilute mineral acids, and in yielding other monosaccharides as well as glucose. They are soluble in Schweitzer's reagent after treatment for a short time with dilute hydrochloric acid. They form a very indefinite group of substances and require further investigation.

Gums. Pectins. Mucilages.

The gums, pectins and mucilages are complex polysaccharides containing both hexose and pentose units. The gums appear to be carbohydrates combined with acids; some are completely soluble, others are partially soluble in water and others only swell up with water.

Mucilages are very widely distributed in plants and form a slimy liquid with water.

Pectins are contained in fruits, turnips, etc. The gelatinisation of boiled fruit extracts is probably due to the presence of pectin.

Schryver and Haynes [1] showed that these plant materials contained the acid substance, pectinogen, which is soluble in water. Pectinogen is readily changed into another acid substance, pectin, by dilute alkali. Mineral acids precipitate pectin as a gel from the alkaline solution; calcium chloride gives a gelatinous precipitate of the calcium salt. Pectin has the composition $C_{17}H_{24}O_{16}$ and contains a pentose group. Pectinogen is extracted from the pressed residue of the plants by warm 0·5 per cent. ammonium oxalate solution.

GLUCOSIDES.

In addition to the carbohydrates there also occur in nature a large number of compounds which contain glucose, more rarely other sugars, e.g. galactose, rhamnose and disaccharides, combined with other organic compounds, especially those belonging to the aromatic series. These are the glucosides.

Glucosides have also been prepared in the laboratory from glucose, mannose, maltose, etc. Two isomers are generally thus obtained, termed the α- and β-glucosides. The chief of the synthetical glucosides are the α- and β-methyl glucosides, which are prepared by the action of hydrochloric acid upon a solution of glucose in methyl alcohol. They are derived from α- and β-glucose by the replacement of the hydrogen atom in the hydroxyl group attached to the carbon atom which possesses aldehydic properties :—
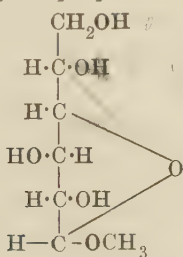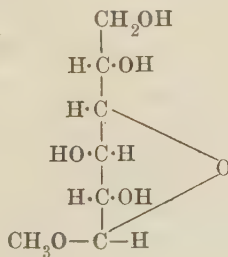
α-methyl glucoside.   β-methyl glucoside.

[1] Biochem. J., 1916, 10, 539.

These two glucosides, besides having different physical properties, be-have differently towards the enzymes, maltase and emulsin. Maltase hydrolyses the a-glucoside, but not the β-glucoside, emulsin hydrolyses the β-glucoside, but not the a-glucoside. The natural glucosides are, in general, hydrolysed only by emulsin and would be derivatives of β-glucose, i.e. β-glucosides.

The three best-known glucosides are probably

$$C_6H_4\begin{cases} O . C_6H_{11}O_5 \\ CH_2OH \end{cases}$$
Salicin.

$$C_6H_5 . CH\begin{cases} CN \\ O . C_{12}H_{21}O_{10} \end{cases}$$
Amygdalin.

$$C_6H_4\begin{cases} O . C_6H_{11}O_5 \\ OH \end{cases}$$
Arbutin.
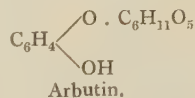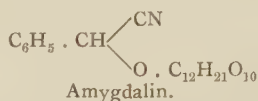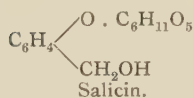
Salicin is a combination of glucose with saligenin, or salicylic alcohol.

Amygdalin is a combination of 2 molecules of glucose, hydrogen cyanide and benzaldehyde.

Arbutin is a combination of glucose with hydroquinone.

The composition of glucosides is ascertained by identification of their products of hydrolysis.

Preparation.

The quantity of glucoside present in plants is usually small. Since enzymes are present which hydrolyse the glucoside, it is advantageous to destroy the enzyme by heating with water, or alcohol, before extract-ing the glucoside. The glucoside is usually isolated by extracting the material with water, alcohol, ethyl acetate, or other organic solvent, concentrating the extract and crystallising out the glucoside. In some cases the extract requires purification, so that no general scheme can be given for isolating glucosides.

Properties.

The glucosides are usually white crystalline substances, soluble in water and having a bitter taste. They are soluble in some organic solvents, but generally insoluble in ether.

Reactions of Salicin.

(1) Salicin does not reduce Fehling's solution.

(2) Salicin solutions are hydrolysed by boiling with dilute sul-phuric acid into glucose and salicylic alcohol. The solution, after neutralisation with soda, reduces Fehling's solution.

Reactions of Amygdalin.

(1) Solutions of amygdalin do not reduce Fehling's solution.

(2) Solutions of amygdalin are hydrolysed by boiling with dilute nitric acid into benzaldehyde, hydrogen cyanide and glucose. The solution smells of benzaldehyde and hydrogen cyanide. The presence

of hydrogen cyanide may be shown by testing with silver nitrate ; the presence of glucose by neutralising with soda and testing with Fehling's solution.

Other glucosides are known which also contain hydrogen cyanide. They are generally referred to as cyanogenetic glucosides. Their presence in leaves may be detected by chewing a small piece of the material, or better by introducing the bruised material and a drop of chloroform into a small test tube, hanging a piece of picric acid test paper [1] in it and closing it with a cork. Hydrogen cyanide is slowly evolved and it colours the test paper orange-red.

[1] This is prepared by dipping strips of filter paper into a 1 per cent. solution of picric acid, drying them, wetting them with a 10 per cent. solution of sodium carbonate and again drying.

CHAPTER XXIX.

ESTIMATION OF CARBOHYDRATES.

THE methods of estimating carbohydrates depend ultimately on the methods of estimating glucose. Though at first sight the estimation of glucose may appear as a comparatively easy task, yet on examination of the literature few subjects seem to have been more worked at than this simple problem. Over thirty methods have been devised by the most distinguished chemists and new ones are continually being described and advocated.

Three of the properties of glucose (and other carbohydrates) are most usually made use of for its estimation :—

A. Its optical activity, by means of the polarimeter.

B. Its aldehyde character, by the reduction of metallic salts, especially copper.

C. Its fermentation, by yeast.

Each of these methods has its own particular advantages, which depend mainly upon its convenience, ease of manipulation, rapidity of completion, and desired accuracy.

A. ESTIMATION BY MEANS OF THE POLARIMETER.

1. The Construction of a Polarimeter.

In an ordinary ray of light the vibrations of the waves take place in all planes perpendicular to the direction of its propagation. If such a ray of light be passed through a crystal of Iceland- or calc-spar and an object be observed through the crystal, two images will be seen. The ray of light has been split into two rays, one of which has been more refracted than the other. The more refracted, or ordinary, ray travels through the crystal just as it would travel through glass and obeys the laws of refraction. The less refracted, or extraordinary, ray does not obey the ordinary laws of refraction, and it shows a movable image when the crystal is rotated. Both of these rays in their passage through the crystal have been polarised in two directions at right angles to each other : i.e. the vibrations of each ray which are transmitted are now only in one plane.

By employing a rhombohedron of Iceland-spar, cutting it across through its obtuse angles, polishing the cut surfaces, cementing together these cut surfaces with Canada balsam, and blackening the longer sides, a prism is obtained. On passing light through this prism, the ordinary ray is totally reflected by the cut surfaces and absorbed by the blackened side, whilst the extraordinary ray passes through and emerges in a direction parallel to the

source of the light.   Such a prism is termed, after its discoverer, a Nicol prism.

In a polarimeter two Nicol prisms, mounted in line with one another, are employed.   The first is fixed, the second is capable of being rotated.   Light is passed through the first prism (the polariser) and reaches the second prism (the analyser).   If this second prism be exactly parallel to the first, the beam of light will also pass through it ; if it be not exactly parallel but inclined at an angle, less light will pass through it : if the second prism be at right angles to the first, or crossed, the light is entirely cut off.

By interposing between the prisms, set parallel to one another, a solution of an optically active substance, the amount of the light is diminished, but it can be brought to its original intensity by rotating the analysing prism.   The amount of rotation necessary to effect this corresponds with the power of rotation of the solution.   As the analysing prism is mounted on a graduated circle, the number of degrees rotated can be measured.   This is the rotatory power of the solution.

The determination of equal illumination of light in such an instrument before and after its passage through an optically active solution is very difficult and the readings are erroneous.   Several devices have been adopted to overcome this difficulty, the simplest being that of Laurent.   Laurent placed behind the polariser a quartz plate of special thickness and of such a size that it covered half the field.   This quartz plate divides the ray of light passing through it into two rays, one of which is retarded by half a wave length and therefore reversed in direction, whilst the other is unaffected. The resultant ray formed on emergence by their fusion will be vibrating in a plane at an angle to the original plane, i.e. the polarised light passing through the quartz plate is rotated through a certain angle.   Thus, if AO be the original plane before passage through the quartz plate, it is resolved into AC and AD. Supposing AD is retarded and reversed, then the components AC and AD′ will form the resultant plane AR.   The angle CAO = angle CAR.
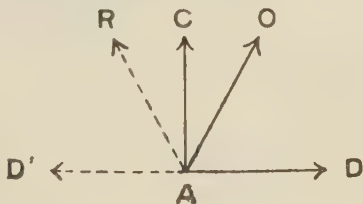
Two beams of polarised light at an angle to one another will therefore reach the analyser.   If the analyser be set parallel to the beam AO arriving from the uncovered portion, this half of the field will appear light and the other half will appear dark.   If it be set parallel to the beam AR coming from the covered portion, this half of the field will appear light and the other half dark.   By adjusting the analyser, a position will be found where the two halves will appear equally illuminated.   This position is the zero-point.

The two halves of the field are illuminated by component portions of the two beams.   At the zero point the two prisms are almost in a crossed position. The instrument is most sensitive under these conditions, but the amount of light is at a minimum.

In other polarimeters, such as Lippich's, a prism which has the same effect as a quartz plate is placed in the centre of the field.   The centre and sides of the field appear dark, or light.

In determinations with such polarimeters monochromatic light must be used ; for convenience, sodium light is generally used, and in this case a cell containing potassium bichromate is introduced in front of the polariser to cut off blue rays ; green light from a mercury lamp is sometimes used.

A polarimeter (Figs. 35, 36) will thus consist of a bichromate cell, a polar-

ising prism, a quartz plate over half the field, a trough to take the solution to be examined, an analysing prism mounted in a movable circle graduated in degrees. There is, in addition, a telescope to focus the edge of the quartz
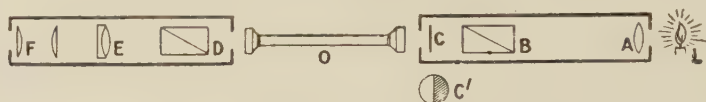
FIG. 35.

L = source of light.   A = lens to render rays of light parallel.   B = Polarising prism.
C, C′ = quartz plate.   O = observation tube.   D = analysing prism.   E, F = telescope.
(From Findlay's " Practical Physical Chemistry.")

plate and a double vernier on each side of the circle in which the analyser is mounted.   This vernier is fixed and graduated in fractions of a degree, or in minutes.
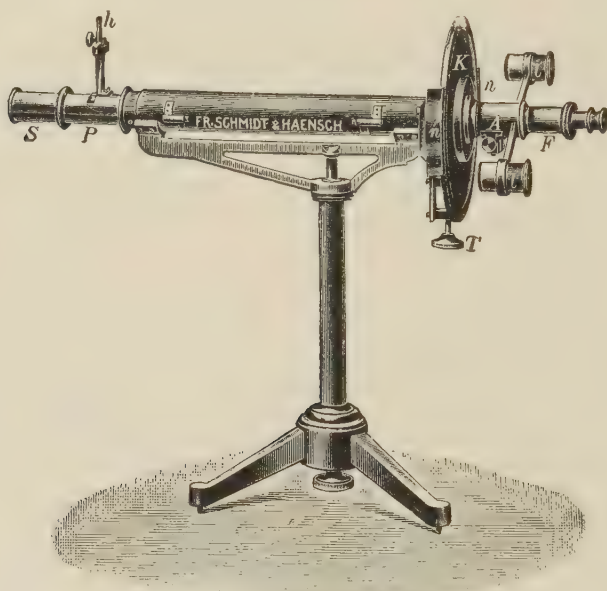
FIG. 36.

At S, lens and bichromate cell.   At P, polarising prism.   At h, lever to rotate polarising prism.   At A, analysing prism which can be rotated by a screw.   At F, telescope with eye-piece.   K = graduated scale.   n, n′ = fixed verniers.   T = screw for rotating graduated scale.   l = magnifying lens to read scale and verniers.

2. The Observation Tube.

The solution of the substance is placed in a special observation tube (Fig. 37). These tubes are generally 0·5, 1, 2, or 2·2 decimetres long ; they are made of glass of the exact length ; the ends are closed by cover glasses held in place by a screw cap and rubber washer. Very small tubes for use with small amounts of solution are also made.

These tubes are thoroughly dried by pushing a plug of filter paper

through them, or thoroughly washed by rinsing several times with the solution under examination. The cover glasses must be dry and without serious scratches. One end of the tube is closed by a cover glass, brass cap and washer, and the solution is filtered into it at the other end until a meniscus just projects above the opening. A short time is given to

FIG. 37.

allow air bubbles to rise. The other cover glass is slid horizontally over the end of the tube, so that it pushes off the excess of liquid and exactly covers the end leaving no air-bubbles underneath it and no liquid on its upper surface. The brass cap and washer is then screwed down over it. The brass caps must not press too tightly on the glass covers.

3. Reading the Polarimeter.

At the point of equal illumination of the two halves of the field the zero of the circular scale coincides, or very nearly coincides, with the zero of the vernier. The exact position must be determined. When an optically active substance is placed between the prisms and equal illumination of the two fields restored, the circular scale will have moved in a clockwise direction (= dextrorotation), or in a counter clockwise direction (= laevorotation), from the vernier. The distance apart of the two zeros measured on the circular scale gives the amount of rotation in degrees; the fraction, or minutes, more is given by the vernier scale.

Several observations of the zero point of the instrument and then several of the solution must always be made. The mean of each is taken and the difference gives the rotation.

4. Estimation.

As the rotatory powers of all the common optically active compounds have now been determined, use can be made of these values to determine the strength of an unknown solution. These values are expressed as specific rotatory power, i.e. the rotation of 1 gm. of substance in 1 c.c. of liquid examined in a layer 1 decimetre (10 cm.) long, i.e. it is the rotatory power of a 100 per cent. solution. This has not actually been carried out, but it has been calculated from the rotations of exactly known strengths of solution. The symbol $[a]_D$ is used to express this value, the D standing for sodium light. The rotation varies with the temperature of the solution and is also recorded; this reading is included in the

symbol, thus $[a]_D^{20}$.   Rotations are generally measured at 20°, but may be taken at other temperatures.

The following are the values for the principal sugars in solutions containing about 10 per cent :—

Mannose = + 14·2°	Lactose = + 52·5°	Glycogen = + 196·6°
Glucose = + 52·7°	Maltose = + 138°	Dextrin = + 195°
Fructose = − 93°	Sucrose = + 66·5°	Starch = + 199°
Galactose = + 83°	Raffinose = + 104°	

The strength of the solution is then given by the formula :—

$$[a]_D = \pm \frac{a \times 100}{c \times l}$$

in which $[a]_D$ = specific rotation.
a = observed rotation.
c = concentration.
l = length of tube in decimetres.

B. ESTIMATION BY REDUCTION OF COPPER SALTS.

The estimation of glucose, and other carbohydrates, is most commonly and most easily effected by means of the reduction of copper sulphate in alkaline solution.   The original method, and still the most convenient method, is that of Fehling-Soxhlet, commonly called Fehling's method.   The other methods are in reality variations of this method, useful in special conditions.

(1) The Fehling-Soxhlet Method.

The method depends upon the complete reduction of the cupric sulphate in Fehling's solution to cuprous oxide, as shown by decolorisation of the solution.

The great difficulty of the estimation is the determination of the end point, i.e. when the blue colour is completely discharged.   The eye by itself is not very sensitive, but the first trace of yellow in the solution can generally be seen.   When the sugar solution is added finally 0·1 c.c. at a time, this amount, or 0·2 c.c., can be deducted, depending on the observer's judgment.

Several indicators have been suggested to help in determining the end point.   They are not necessary, except in the case of coloured sugar solutions. The eye, after a little practice, will see the exact point of decolorisation, or a slight tinge of yellow.

The estimation should be carried out in three stages :—

(a) Preliminary Rough Estimation.

10 c.c. of Fehling's solution (i.e. 5 c.c. of each) are measured out with a pipette into a porcelain basin, or small flask, diluted with about 40 c.c. of water, and raised to the boiling-point.

The Fehling solution is now kept *gently boiling* the whole time, and the sugar solution is run in from a burette, 1 c.c. at a time.

The reduction must be allowed to complete itself before adding a fresh quantity of the sugar solution.

It is noted when the blue colour of the solution has entirely disappeared. The solution may become slightly yellow, due to the action of the alkali of the Fehling's solution upon excess of the sugar solution.

An idea of how much sugar is present in the solution is thus obtained, i.e. the amount will be between 1 and 2, 2 and 3, 3 and 4 c.c., etc.

(*b*) **Dilution, or Concentration, of the Sugar Solution.**

Since the method is only accurate if the concentration of the sugar is between 0·5 and 1 per cent., the sugar solution must be diluted, or concentrated.

It is best to have as nearly as possible 10 c.c. of the diluted, or concentrated, sugar solution = 10 c.c. of Fehling's solution. If less than 10 c.c. of the glucose solution have been used, a known volume of the solution is diluted ; if more, a known volume is concentrated to a smaller volume, e.g. 100 c.c. to 30 c.c.

Suppose 3 c.c. of the sugar solution were sufficient ; then 3 c.c. should be diluted to 10 c.c. It is more convenient to dilute a larger quantity : 30 c.c. are measured out with a pipette into a 100 c.c. measuring flask, the flask is filled to the mark with water and the contents are mixed : or 30 c.c. are measured into a dry flask and 70 c.c. of water are added with a clean pipette.

The burette is carefully rinsed out with the diluted sugar solution and the final titration carried out.

(*c*) **Final Titration.**

10 c.c. of Fehling's solution are diluted as before with 40 c.c. of water and the diluted glucose solution carefully added to the boiling liquid. It is advisable to run in at once a little less than the amount required to decolorise the solution entirely (say 8 c.c.), and then to add cautiously 0·1-0·2 c.c. at a time until there is complete decolorisation, always allowing time for the reduction to occur. This final titration should be repeated running in practically all the glucose solution necessary at one time, and then completing with 0·1 c.c. at a time. Suppose 10·1 c.c. were insufficient, but 10·3 c.c. too much, as seen by a faint yellow coloration of the solution, then 10·2 c.c. is the proper value.

Soxhlet carried out altogether 5 or 6 titrations, adding more or less than the exact amount of glucose solution at once, and thus determined the limits of too much and too little until they approached one another and differed by only 0·1 c.c.

The Determination of the End Point. Use of Indicators.

Several indicators have been suggested to show the exact end point, e.g. potassium ferrocyanide, ferrous thiocyanate, starch and potassium iodide, reduced phenolphthalein.   The most useful is perhaps starch and potassium iodide, as suggested by E. F. Harrison in 1903.   Its use depends upon the liberation of iodine by cupric salts and it will show the presence of copper sulphate in a dilution of 1 in 20,000.

A drop of the titration solution is added to 1 c.c. of the indicator acidified with 10 drops of acetic acid.   A red, or blue, colour is shown, if cupric salt be present ; no colour is given when reduction is complete.

Lavalle has suggested that the dilution of the Fehling's solution be done with caustic soda solution instead of with water.   The cuprous oxide either settles better, or stays in solution, depending on the amount used ; but the result is not so accurate in the presence of excess of caustic soda.

Calculation of the Result.

Knowing the dilution, the amount of sugar in the original solution can be calculated :—

<div align="center">

10 c.c. Fehling's solution = 0·05 gm. glucose,

∴ 10·2 c.c. diluted sugar solution = 0·05 gm. glucose.

Now 100 c.c. diluted sugar solution contain 30 c.c. original sugar solution,

∴ 10·2 c.c. diluted sugar solution contain $\dfrac{30 \times 10\cdot2}{100}$ c.c. original sugar solution,

∴ $\dfrac{30 \times 10\cdot2}{100}$ c.c. original solution = 0·05 gm. glucose,

∴ 100 c.c. original sugar solution = $\dfrac{100 \times 100 \times \cdot05}{30 \times 10\cdot2}$

= 1·6 per cent.

</div>

The values of Fehling's solution for other monosaccharides are almost the same as glucose, thus 10 c.c. = ·05 gm. glucose = ·0511 gm. galactose = ·05144 gm. fructose = ·0431 gm. mannose.

(2) S. G. Benedict's Method.

S. G. Benedict has introduced a modification of the Fehling method which overcomes most of the difficulties and inconveniences.   The method depends upon the precipitation of the reduced copper as white cuprous thiocyanate and decolorisation of the solution.

If potassium thiocyanate be added to Fehling's solution, the cuprous oxide is not precipitated on reduction, but if carbonate be used instead of caustic alkali, a white precipitate of cuprous thiocyanate is formed.

Procedure.

25 c.c. of the reagent are measured with a pipette into a porcelain basin, 25-30 cm. in diameter ; 10-20 gm. of cryst. sodium carbonate

(or 5-10 gm. anhydrous sodium carbonate) and a small quantity of pumice, or a piece of porous earthenware, are added. The solution is boiled vigorously over a free flame and the sugar solution is run in rapidly till a heavy white precipitate is produced and the blue colour begins perceptibly to diminish. The sugar solution is then run in more slowly with constant vigorous boiling of the reagent until the blue colour has entirely disappeared. An interval of 30 seconds between the additions of sugar solution (drop by drop) towards the end should be given, and water may be added to replace that lost by evaporation. The sugar solution should be of 0·5-1 per cent. as in Fehling's method.

The calculation of the result is from

25 c.c. reagent = 0·05 gm. glucose or ·053 gm. fructose.

Sometimes the end point is not exact decolorisation, but a greenish colour.

(3) Pavy's Method.

Owing to the difficulty experienced in determining the end point in Fehling's method, especially in the case of the estimation of glucose in liquids, such as urine, owing to the formation of ammonia, which prevents the precipitation of cuprous oxide (cf. p. 259), the use of ammonium salts was introduced by Monier. A practical method for estimating glucose was worked out by Pavy.

Procedure.

50 c.c. of Pavy's solution are measured with a burette into a 200 c.c. conical flask. The flask is closed by a cork with two holes; through one of these the end of the burette containing the sugar solution is passed and through the other an escape tube to carry off steam and ammonia. To prevent the ammonia fumes coming into the air, Pavy fitted to the escape tube a U-tube containing pumice and sulphuric acid, but it is most convenient to fit a valve as described by Allen. This consists of a short length of rubber tubing closed at its end by a piece of glass rod and cut near the end with a V-shaped slit. This arrangement is preferable to the valve described in 1904 by Kumagawa and Suto. The end of the valve is placed in a dilute solution of sulphuric acid, which is renewed when the acid is neutralised.

The solution is boiled to drive out the air, which readily oxidises ammoniacal cuprous solutions, and the sugar solution (0·5 to 1 c.c. at a time) is gradually run in until the blue colour is discharged, the solution being kept boiling throughout to exclude air. Sufficient time must be allowed for the reduction to take place, as it is slower than with Fehling's solution. The valve prevents any liquid being sucked back, if the sugar solution be run in so quickly that boiling is stopped.

Just as in Fehling's method, the sugar solution must be of such a strength that 10 c.c. = 50 c.c. of Pavy's solution. The titration must be carried out rapidly and must be completed *within three minutes*, otherwise the ammonia is all evolved before the titration is completed and cuprous oxide is deposited. The boiling must not be interrupted and the sugar solution must be run in at such a rate that the solution is kept boiling the whole time. The final

estimation should be made by running in rather less ('5 c.c.) than the amount required and finishing off more slowly. The minimal amount which is found to reduce the Pavy's solution completely, when added at one time, is the exact volume of the solution required.

The amount of sugar in the solution is calculated from

$$\text{10 c.c. Pavy's solution} = \text{0·005 gm. glucose.}$$

The method has been compared against other methods by Kinoshita who finds it very accurate.

(4) Gerrard's Method.

In 1892 Gerrard found that potassium cyanide was an effective agent for dissolving cuprous oxide and prevented its precipitation from Fehling's solution when reduced by glucose. This observation led to a simple method for estimating glucose. It was improved by Allen and described by him as the best method. The method has an advantage over Pavy's method in the absence of the ammonia vapour and in that the reoxidation of the cuprous oxide is slower.

On adding potassium cyanide to Fehling's solution it is decolorised, the colourless double salt of copper and potassium cyanide being formed :—

$$CuSO_4 + 4KCN = CuCN_2, 2KCN + K_2SO_4.$$

If excess of Fehling's solution above that capable of being decolorised be added the blue colour remains, and when boiled with glucose this amount is reduced without the precipitation of cuprous oxide.

Allen described the following procedure :—

10 c.c. of Fehling's solution are diluted with 40 c.c. of water and heated to boiling in a porcelain basin. An approximately 5 per cent. solution of potassium cyanide is run into the boiling liquid from a burette until it is just decolorised, excess being carefully avoided. Another 10 c.c. of Fehling's solution are added and the sugar solution of about 0·5 per cent. strength run in slowly until the blue colour vanishes. Only the last portion of the Fehling solution is reduced by the glucose so that, as in Fehling's method, 10 c.c. = 0·05 gm. glucose.

C. ESTIMATION BY FERMENTATION.

Sugar is sometimes estimated by fermentation with yeast in an Einhorn fermentation tube. This is a U-shaped tube, one limb of which is closed and the other expanded into a bulb (Fig. 38).

Fig. 38.

The closed limb of these tubes is filled with the sugar solution to which some yeast has been added. Mercury is placed at the bend. The carbon dioxide evolved collects in the closed limb and drives down the solution into the other and wider limb. The narrow limb is graduated in percentages of glucose, so that the amount of sugar can be directly read off.

This fermentation method is not very accurate and is consequently not often used for estimating sugar.

Another method of estimation is by taking the specific gravity of the solution before and after fermentation.

The most accurate results by fermentation are obtained with Lohnstein's apparatus (Fig. 39).

A U-shaped tube is employed. The straight limb is left open, but the bulb is closed, after filling, with a stopper. The straight limb is narrower at the base than at the upper end, and upon the end rests a wooden scale graduated in percentages of glucose ; the graduations on one side are for working at room temperature, on the other side at 37°.

A definite weight of mercury is placed in the bend ; it almost fills the bulb and reaches up the narrow part of the limb to about the zero mark on the scale. Upon the surface of the mercury in the bulb is placed 0·5 c.c. of glucose solution and 1-2 drops of a suspension of yeast in water. The stopper (carefully greased) is inserted. It is perforated with a small hole which coincides with a similar hole at the neck of the bulb. With the two openings together so that air can enter, or be displaced, the apparatus is tilted so as to put the mercury in the limb at the zero point of the scale. The stopper is then turned to close the apparatus and it is set aside for the fermentation to proceed for 12-24 hours at room temperature, or for 3-4 hours at 37°. To prevent the stopper being forced out by the pressure of the gas it is covered with a weight.

FIG. 39.

The percentage of glucose is read off on the scale when the fermentation has ended and the apparatus has returned to room temperature, if the fermentation took place at 37°. Lohnstein shows that a more accurate result is obtained if the readings are taken at 37° and 20° and if the percentage is calculated from an equation.

The apparatus should be cleansed immediately after use ; the opening on the stopper is slowly made to coincide with the opening of the bulb. The mercury level takes the original position. The liquid is removed with a small pad of cotton wool, and the surface of the mercury washed with a little water, which is drained away by fresh cotton wool.

ESTIMATION OF PENTOSES.

Like the hexoses, the pentoses reduce Fehling's solution and can be estimated in the same way, if they are present in the solution alone ; generally a mixture of pentose and other carbohydrates is present ; under these conditions the pentose can be estimated by converting it into furfural (p. 329)

by distillation with hydrochloric acid; and combining the furfural with phloroglucinol and weighing the compound.

ESTIMATION OF DISACCHARIDES.

A. Cane Sugar.

Cane sugar is estimated by taking the reducing power of the solution after hydrolysis by acid; fructose and glucose have very nearly the same reducing power.

A known volume of the cane sugar solution (say 40 c.c.) is hydrolysed by warming on the water-bath for 5-10 minutes with 5 c.c. of dilute sulphuric acid. The solution is cooled, neutralised with 5 c.c. of caustic soda, and made up to a definite volume in a measuring flask (say 100 c.c.), rinsing out the flask with the water necessary to make up the 100 c.c. The amount of reducing sugar is then estimated by Fehling's, or any of the other methods previously described. The sugar solution should be of a strength so that 10 c.c. = 10 c.c. Fehling's, i.e. contain about the equivalent of ·5 per cent. of glucose.

The percentage of cane sugar is calculated from the equation :—

$$\underbrace{C_{12}H_{22}O_{11}}_{\substack{342 \\ 0\cdot047}} + H_2O = \underbrace{2C_6H_{12}O_6}_{\substack{360 \\ 0\cdot05 \ = \ 10 \ \text{c.c. Fehling's solution.}}}$$
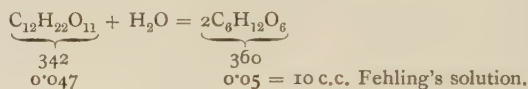
B. Lactose and Maltose.

Lactose and maltose are estimated in the same way as glucose by Fehling's or other methods, but their reducing power is less than that of glucose. Consequently, since Fehling's solution is a standard for glucose, a factor has to be employed in order to obtain the equivalent value for these disaccharides.

The factor is obtained by determining the reducing power before and after hydrolysing them into monosaccharides by acid, thus :—

A definite volume of the lactose solution is taken and diluted, as previously done for glucose, with water so that 10 c.c. reduce 10 c.c. Fehling's solution. The value is determined exactly.

Exactly the same quantity of the lactose solution is hydrolysed by boiling for 3-4 hours in a flask with one-tenth of its volume of dilute sulphuric acid; water must be added to replace that lost on boiling, or the hydrolysis is carried out by heating under a reflux condenser. The solution is cooled, neutralised with soda, and made up to the same volume as the non-hydrolysed lactose solution with water as above.

It is best to make up the solutions in a measuring flask, e.g. 25 c.c. lactose solution are diluted to 100 c.c.; 25 c.c. lactose solution are hydrolysed by acid, neutralised, and washed into a 100 c.c. measuring flask.

The reducing value of the hydrolysed lactose solution is taken exactly.

The factor is $\dfrac{\text{reducing power of lactose in c.c.}}{\text{reducing power of hydrolysed lactose in c.c.}} = \dfrac{10}{7}$.

The ratio of the reducing powers of glucose : lactose : maltose are as 1 : 0·74 : 0·62.

The percentage of lactose may be calculated as follows :—

$$x \text{ c.c.} = 10 \text{ c.c. Fehling's solution.}$$

$$\therefore x \times \frac{7\cdot4}{10} \text{ c.c.} = 0\cdot05 \text{ gm. lactose,}$$

$$\text{or } x \text{ c.c.} = 0\cdot05 \times \frac{10}{7\cdot4} \text{ gm. lactose,}$$

$$\text{Hence 100 c.c.} = 0\cdot05 \times \frac{10}{7\cdot4} \times \frac{100}{x} \text{ gm. lactose.}$$

The weights of the disaccharides which will reduce completely 10 c.c. of Fehling's solution are :—

cane sugar	0·0475 gm.
maltose	0·0807 ,,
lactose	0·0678 ,,

ESTIMATION OF POLYSACCHARIDES.

All polysaccharides are estimated in the same way as cane sugar, i.e. by taking the reducing value of a known weight, or volume, of the solution after hydrolysis by acid, i.e. in terms of glucose :—

$$C_6H_{10}O_5 + H_2O = C_6H_{12}O_6.$$

CHAPTER XXX.

HIGHER AMINES, DIAMINES, COLAMINE, CHOLINE, BETAINES.

DURING the putrefaction of proteins certain higher amines, and the two diamines, putrescine and cadaverine, are produced. Colamine and choline are present in the lecithines, or lipines. Betaines are found in various plants.

Higher Amines.

The higher amines are produced from the corresponding amino acid by loss of carbon dioxide through the action of putrefactive bacteria :—

$$\begin{array}{l} CH_3 \\ | \\ CH . NH_2 \\ | \\ COOH \\ \text{Alanine.} \end{array} = CO_2 + \begin{array}{l} CH_3 \\ | \\ CH_2 . NH_2 \\ \\ \text{Ethylamine.} \end{array}$$
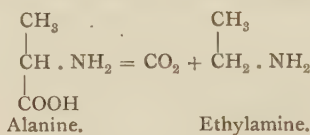
Similarly, valine gives isobutylamine,

leucine gives isoamylamine,

isoleucine gives methylethyl-ethylamine :—

$$\begin{array}{l} CH_3 \diagdown \\ \qquad CH . CHNH_2 . COOH \\ C_2H_5 \diagup \end{array} = CO_2 + \begin{array}{l} CH_3 \diagdown \\ \qquad CH . CH_2 . NH_2. \\ C_2H_5 \diagup \end{array}$$

These compounds are primary amines and closely resemble methyl-amine.

Diamines.

The diamines correspond with the dihydroxy alcohols, containing the amino groups upon different carbon atoms. The first member of the group, methylene diamine $CH_2(NH_2)_2$, is unknown, except in the form of derivatives. It appears as if two amino groups, like two hydroxyl groups, cannot exist attached to the same carbon atom.

Ethylene diamine is formed by the action of ammonia upon ethylene dibromide.

$$\begin{array}{l} CH_2Br \\ | \\ CH_2Br \end{array} + 2NH_3 = \begin{array}{l} CH_2 . NH_2 \\ | \\ CH_2 . NH_2 \end{array} + 2HBr.$$
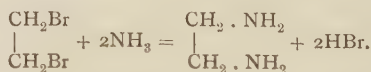
Putrescine, or tetramethylene diamine, is formed from ornithine, and arginine, by putrefaction :—

$$H_2N . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH = CO_2 + H_2N . CH_2 . CH_2 . CH_2 . CH_2 . NH_2$$

Putrescine can be synthesised from ethylene dicyanide :—

$$\begin{array}{ccccccc}
CH_2 & & CH_2Br & & CH_2CN & & CH_2 . CH_2 . NH_2 \\
\| & \rightarrow & | & \rightarrow & | & \rightarrow & | \\
CH_2 & & CH_2Br & & CH_2CN & & CH_2 . CH_2 . NH_2.
\end{array}$$
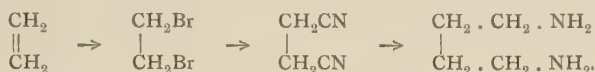
Cadaverine, or pentamethylene diamine, is formed from lysine in the same way :—

$$H_2N . CH_2 . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH =$$
$$CO_2 + H_2N . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . NH_2.$$
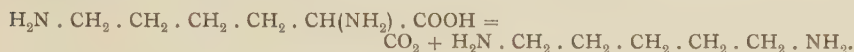
Cadaverine can be prepared from allyl bromide :—

$$\begin{array}{ccccccc}
CH_2 & & CH_2Br & & CH_2 . CN & & CH_2 . CH_2 . NH_2 \\
\| & & | & & | & & | \\
CH & \xrightarrow{HBr} & CH_2 & \xrightarrow{KCN} & CH_2 & \xrightarrow{H_2} & CH_2 \\
| & & | & & | & & | \\
CH_2Br & & CH_2Br & & CH_2 . CN & & CH_2 . CH_2 . NH_2.
\end{array}$$
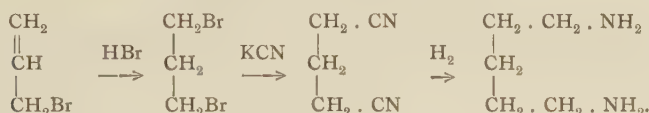
The diamines are very strong bases and absorb carbon dioxide from the air. They closely resemble the simple amines in their chemical properties. Putrescine and cadaverine are of further interest in that they are easily converted into pyrrolidine (p. 332) and piperidine (p. 335).

Colamine, or amino-ethyl alcohol, is a constituent of kephaline (p. 245). It is prepared by the action of ammonia upon ethylene oxide :—

$$\begin{array}{c}
CH_2 \\
| \quad\diagdown \\
\quad\quad O + NH_3 = \\
| \quad\diagup \\
CH_2
\end{array}
\quad
\begin{array}{c}
CH_2OH \\
| \\
CH_2NH_2
\end{array}$$
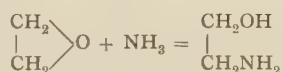
Amino-ethyl alcohol is an oil with peculiar smell, which fumes in the air. It boils at $171°$ at 757 mm. and can be separated from alkaline solution by fractional distillation. It forms double salts with platinum and gold chlorides.

Choline.

Choline, or hydroxy-ethyl-trimethyl-ammonium hydroxide is a trimethylamine derivative of ethyl alcohol,

$$\begin{array}{c}
CH_2OH \\
| \\
CH_2—N : (CH_3)_3 \\
| \\
OH.
\end{array}$$
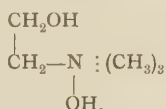
It occurs in the free state in most animal tissues and is widely distributed in plants. It is a constituent of lecithine, which is present in egg yolk, nervous tissue, and in seeds of plants from which it arises by hydrolysis.

Preparation.

Choline is usually prepared from egg yolk. The lecithine is extracted with ether, or alcohol. The residue left on evaporation of the solvent is hydrolysed by boiling with baryta. The barium soaps, which are formed, are filtered off and the solution evaporated to dryness. The choline is extracted from the dry residue with alcohol and precipitated as double salt with mercuric, or

platinum, chloride, from which its hydrochloride is obtained by decomposition with hydrogen sulphide.

Synthetically, choline has been prepared by the action of trimethylamine upon ethylene oxide :—

$$\begin{matrix} CH_2 \\ | \\ CH_2 \end{matrix} \Big> O + (CH_3)_3 : N + H_2O = \begin{matrix} CH_2OH \\ | \\ CH_2-N(CH_3)_3 \\ | \\ OH \end{matrix}$$
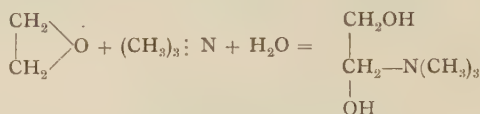
Properties.

Choline is a hygroscopic crystalline mass which has an alkaline reaction. It forms salts with acids, such as choline hydrochloride,

$$\begin{matrix} CH_2OH \\ | \\ CH_2-N(CH_3)_3 \\ | \\ Cl \end{matrix}$$
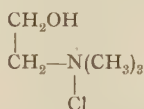
and these salts form double salts with the salts of heavy metals.

Choline has no peculiar reactions and must be isolated and analysed in the form of its salts for its identification. It is decomposed by boiling with alkalies yielding trimethylamine and glycol. On oxidation, it yields betaine.

Esters of Choline.

As an alcohol, choline forms esters with acids :—

(1) Acetyl choline, $\begin{matrix} CH_2O-OC \cdot CH_3 \\ | \\ CH_2-N(CH_3)_3 \\ | \\ OH, \end{matrix}$

has been found in ergot.

(2) Palmityl choline, $\begin{matrix} CH_2O-OC \cdot C_{15}H_{31} \\ | \\ CH_2 \cdot N(CH_3)_3 \\ | \\ OH, \end{matrix}$
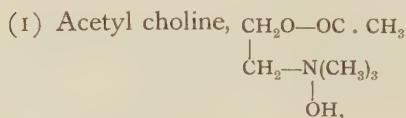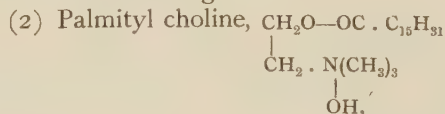
has also been prepared. It is easily soluble in water and readily hydrolysed by alkali. It has a powerful hæmolytic action (Fourneau and Page).

(3) Choline nitrite, $\begin{matrix} CH_2O-NO \\ | \\ CH_2-N(CH_3)_3 \\ | \\ OH \end{matrix}$ and choline nitrate, $\begin{matrix} CH_2O-NO_2 \\ | \\ CH_2-N(CH_3)_3 \\ | \\ OH \end{matrix}$
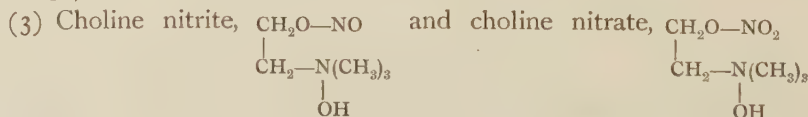
resemble the toxic constituents of the toad-stool (*Amanita agarica*).

The nitrite is formed by the action of nitric acid on choline and was formerly supposed to be the aldehyde (pseudomuscarine or synthetical muscarine),

$$\begin{matrix} CHO \\ | \\ CH_2 \cdot N(CH_3)_3 \\ | \\ OH. \end{matrix}$$
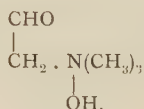
The ethyl ether of choline resembles the action of natural muscarine still more closely (Ewins).   Muscarine is the poisonous base of the toadstool, *Agaricus muscarius*.

Esters of choline have marked pharmacological actions.

Neurine is formed in putrefaction probably from choline by loss of water.

$$CH_2$$
$$\|$$
$$CH\!-\!\!-\!N(CH_3)_3$$
$$|$$
$$OH$$
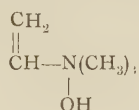
It is prepared from trimethylamine and ethylene bromide and treatment of the product with silver oxide :—

$$\begin{array}{cccc}
CH_2Br & CH_2Br & CH_2 & CH_2 \\
| & | & \| & \| \\
CH_2Br & CH_2 . N(CH_3)_3 & CH . N(CH_3)_3 & CH . N(CH_3)_3 \\
& | & | & | \\
& Br & Br & OH.
\end{array}$$
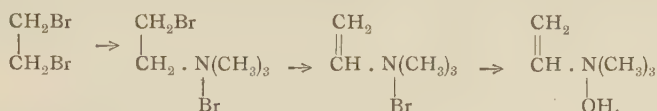
It is very toxic.

Betaine.

Betaine, or trimethylamine acetic acid, was first obtained from the sap of the sugar beet (*Beta vulgaris*), and is present in beet molasses.   It occurs in a large number of plants and has been isolated from shrimp extract and extracts of other invertebrates; ·05 per cent. has been separated from ox kidney.

It is most easily prepared by the action of trimethylamine upon chloracetic acid :—

$$\begin{array}{ccc}
CH_2Cl & & CH_2 . N(CH_3)_3OH \\
| & + (CH_3)_3N & = \qquad | \qquad + HCl \\
COOH & + H_2O & COOH
\end{array}$$

Betaine separates from alcohol in large colourless crystals, which are very soluble in water and have a neutral reaction.   It loses a molecule of water at 100°, or on standing over sulphuric acid.   Its constitution is more probably that of the anhydride,

$$\begin{array}{c}
CH_2 . N(CH_3)_3 \\
| \qquad | \\
CO\!-\!O
\end{array}$$
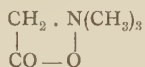
Many other betaines have been isolated from plants.   (See "The Simple Natural Bases," by G. Barger).

GUANIDINE AND ITS DERIVATIVES.

Guanidine.

GUANIDINE was first obtained by the oxidation of guanine (p. 322), and is also a product of the oxidation of arginine (p. 301) with permanganate. Its formation by the oxidation of arginine explains its formation in the oxidation of proteins, which contain arginine. Guanidine has been found in self-digested solutions of pancreas and in extracts of vetch seedlings and in the sap of the beet.

Guanidine is formed by the action of ammonia upon ortho-carbonic ester in a manner similar to the preparation of urea from ethyl carbonate, or carbonic ester (p. 123) :—

$$
C\!\!\begin{cases} OC_2H_5 \\ OC_2H_5 \\ OC_2H_5 \\ OC_2H_5 \end{cases}
\longrightarrow
C\!\!\begin{cases} NH_2 \\ NH_2 \\ NH\,H \\ NH_2 \end{cases}
\longrightarrow
C\!\!\begin{cases} NH_2 \\ \!=\!\!=NH \\ NH_2 \end{cases}
$$

Ortho-carbonic   Hypothetical.   Guanidine.
ester.
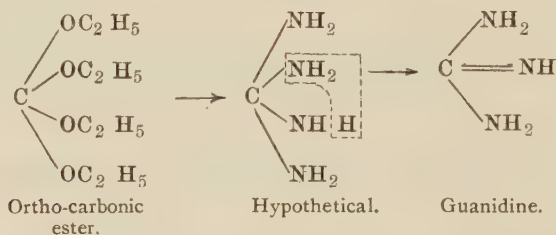
It is generally prepared by the action of ammonia upon cyanamide :—

$$
NH_4SCN \begin{array}{c} \nearrow CS(NH_2)_2 \\ \searrow NH_2.CN \end{array}
$$

$$
NH_4SCN + NH_2CN = C\!\!\begin{array}{c} NH_2 \\ =\!NH \\ NH_2.HSCN. \end{array}
$$
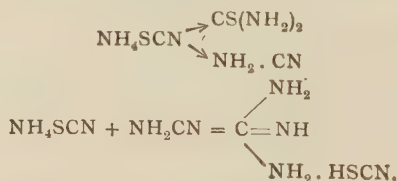
In practice, this reaction is accomplished by heating ammonium thiocyanate to 180-190°. Thiourea and cyanamide are formed. The cyanamide reacts with ammonium thiocyanate to yield guanidine thiocyanate :—

$$
H_2N.C\!\equiv\!N + NH_3 = C\!\!\begin{array}{c} NH_2 \\ =\!NH \\ NH_2. \end{array}
$$
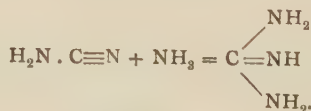
Guanidine is thus urea in which the OH group has been replaced by an amino group.

Guanidine is a deliquescent crystalline substance, easily soluble in water and alcohol. It is a strong base; its solutions have an alkaline reaction and absorb carbon dioxide from the air forming guanidine carbonate, $(CH_5N_3)_2 . H_2CO_3$, which is soluble in water, but not in alcohol. Guanidine also forms salts with other acids; the nitrate, $CH_5N_3 . HNO_3$, is not easily soluble and consists of large plates which melt at $214°$. The chief salt is the picrate which melts at $315°$ and is very insoluble in cold water. This salt is used for the isolation of guanidine from solution and for its estimation. Double salts are formed with gold chloride and cadmium chloride.

Guanidine is hydrolysed by alkalies into urea and ammonia :—

$$\underset{\diagdown NH_2}{\overset{NH_2 \diagup}{C}}{=}NH + H_2O = \underset{\diagdown NH_2}{\overset{OH \diagup}{C}}{=}NH + NH_3,$$
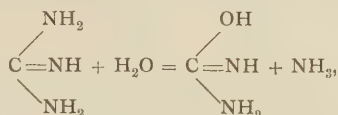
Methyl Guanidine and Dimethyl Guanidine.

$$\underset{\diagdown NH_2}{\overset{NH . CH_3 \diagup}{C}}{-}NH \qquad\qquad \underset{\diagdown NH . CH_3.}{\overset{NH . CH_3 \diagup}{C}}{=}NH$$
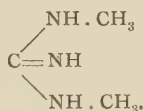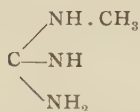
Methyl guanidine has been isolated from meat and meat extracts, in which about ·1 per cent. is present. It has also been isolated from urine.

Dimethyl guanidine has been isolated from urine.

Methyl guanidine is probably derived from creatine.

Arginine, $\quad \overset{NH_2}{\underset{|}{}} \qquad\qquad \overset{NH_2}{\underset{|}{}}$
$$NH{=}C{-}NH . CH_2 . CH_2 . CH_2 . CH . COOH.$$

Arginine, or δ-guanidine-a-aminovalerianic acid is a constituent of the protein molecule; in some proteins—the protamines—it forms over 80 per cent. of the molecule. It was discovered in extracts of seedlings.

Arginine is most easily prepared by the hydrolysis of edestin, the protein of hemp seed,[1] or from seedlings of the yellow lupin.[2] Its constitution was proved by its synthesis from cyanamide and ornithine :—

$$\overset{NH_2}{\underset{|}{CN}} + H_2N{-}CH_2{-}CH_2{-}CH_2{-}\overset{NH_2}{\underset{|}{CH}}{-}COOH =$$
$$\overset{NH_2}{\underset{|}{NH{=}C}}{-}NH{-}CH_2{-}CH_2{-}CH_2{-}\overset{NH_2}{\underset{|}{CH}}{-}COOH.$$
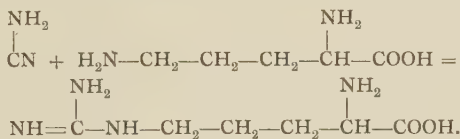
Arginine is a white crystalline substance, easily soluble in water, but insoluble in alcohol. It melts with decomposition at $207·5°$. It is a strong base and absorbs carbon dioxide from the air, forming

[1] See Ber., 1905, **38**, 4187.   [2] See Zeit. Physiol. Chem., 1902, **35**, 314.

arginine carbonate. It also forms salts with other acids, of which the nitrate is the principal one.

Natural arginine is optically active, the synthetical product is optically inactive, but has been separated into its stereoisomers.

Like guanidine, it is hydrolysed by alkalies into urea and ornithine :—

$$\underset{NH_2}{NH=C-NH . CH_2 . CH_2 . CH_2 . CH . COOH} + H_2O =$$

$$NH=C-OH + NH_2 . CH_2 . CH_2 . CH_2 . CH . COOH.$$
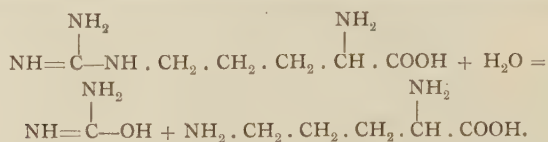
This hydrolysis of arginine occurs in the liver by the action of the enzyme, arginase. A portion of the urea in the urine is probably derived in this way.

$$\textbf{Creatine,} \quad NH=C-N-CH_2 . COOH.$$
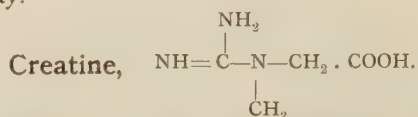
Creatine, or methyl guanidine acetic acid, is a constituent of all vertebrate muscle and is most abundant in voluntary muscle. The creatine content of the muscle of any particular species is remarkably constant; thus ·5 per cent. in rabbit's, ·45 per cent. in cat's, ·39 per cent. in man's, ·37 per cent. in dog's muscle. It is not present normally in human urine, but appears under certain conditions, e.g. when carbohydrates are absent from the food, in diabetes and other diseases. It is present in the urine of infants and children and in that of women after menstruation, and during and after pregnancy. Creatine is normally present in bird's urine.

Preparation.

Creatine is more readily prepared from meat than by synthesis.

Finely minced meat is extracted several times with hot water. The aqueous solution is boiled to remove coagulable proteins and filtered. The filtrate is treated with lead acetate so long as a precipitate is formed and again filtered. Excess of lead is removed from the solution by means of hydrogen sulphide and the filtrate from lead sulphide is evaporated down to a small volume. Creatine crystallises out as the solution stands. It is filtered off and washed with 88 per cent. alcohol.

Creatine has been synthesised from cyanamide and sarcosine, or methyl-glcyine :—

$$C\equiv N + HN . CH_2 . COOH = NH=C-N-CH_2 . COOH.$$
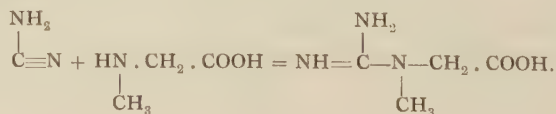
Properties and Reactions.

Creatine separates from water in colourless, transparent, hard rhombic prisms (Fig. 40), containing one molecule of water of crystallisation, which is given off at 100°, the specimen becoming opaque. It has a peculiar bitter taste, is easily soluble in hot water, less so in cold (1 in 74), almost insoluble in alcohol and insoluble in ether. Its solution has a neutral reaction. It forms salts with acids which are very unstable.

It is hydrolysed by alkalies into urea and sarcosine and may, therefore, like arginine, contribute to the quantity of urea in urine :—

$$NH=\overset{\overset{\displaystyle NH_2}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C}}-N-CH_2 . COOH + H_2O = NH=\overset{\overset{\displaystyle NH_2}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C}}-OH + HN . CH_2 . COOH.$$
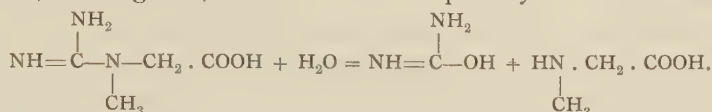
Conversion of Creatine into Creatinine.

Creatine, on heating with acids, is converted into its anhydride, creatinine :—

$$HN=\overset{\overset{\displaystyle NH_2}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C}}-N-CH_2COOH = H_2O + HN=\overset{\overset{\displaystyle NH\text{————}}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C}}-N-CH_2 . CO.$$
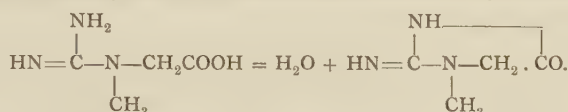
The reverse action takes place when creatinine solutions are heated to boiling, or on boiling with alkalies.

FIG. 40.—Creatine. (After Funke.)   FIG. 41.—Creatinine. (After Funke.)

Creatinine.

Creatinine is present in all mammalian urines. The amount of creatinine in human urine varies from about 0·75-1·5 gm. per diem. It is not present in muscle, or it is present only in traces. It has been found in wheat, rye and other crops, and has been isolated from cultivated soil.

Creatinine is present in meat extracts. Its presence is probably due to the action of the acids of muscle extracts upon creatine during evaporation.

Creatinine is obtained from creatine by boiling with acids. It is most conveniently prepared from human urine.

Properties.

Creatinine separates from hot saturated solutions in colourless, shining prisms (Fig. 41), from cold saturated solutions in plates, or prisms containing $2H_2O$. It has a caustic taste and its solutions react slightly alkaline. It is soluble in 11·5 parts of cold water, more easily in hot water, in 625 parts of cold absolute alcohol and more easily in hot alcohol.

It behaves as a strong alkali, displacing ammonia from its salts. It forms salts with acids and double salts with salts of the heavy metals, of which the zinc chloride double salt is the most characteristic.

Reactions.

Creatinine has two reactions by which its presence in a solution can be detected, e.g. in urine :—

(1) *Sodium Nitroprusside Reaction* (*Weyl*).

A few drops of a dilute freshly prepared solution of sodium nitroprusside are added to a small quantity of the solution (urine) and dilute sodium hydroxide is added drop by drop. The solution becomes red in colour and in a short time changes to yellow. If the yellow solution be acidified with glacial acetic acid and heated, the solution becomes green and a deposit of Prussian blue forms on standing.

Note.—Acetone gives a similar colour reaction, but the colour changes to purple on acidifying. It is advisable to remove acetone, if present, by boiling the solution before testing for creatinine.

(2) *Picric Acid Reaction* (*Jaffé*).

To the solution containing creatinine (urine) some saturated picric acid solution is added and the mixture made alkaline with sodium hydrate. The solution becomes deep orange in colour which remains permanent for some hours.

One part of creatinine in 5000 can be detected by this reaction.

Note.—Aldehyde, acetone and other compounds also reduce picric acid in the cold; glucose, fructose, urea, etc., reduce it on warming (Chapman).[1] The picric acid is converted into picramic acid, amino-dinitrophenol and diamino-nitrophenol.

Estimation of Creatinine.

Folin has shown that creatinine can be accurately estimated by means of Jaffé's reaction. The orange-red colour produced is matched in a colori-

[1] Analyst, 1909.

meter against the colour of ·5N solution of potassium bichromate, or better against a solution of creatinine, or creatinine zinc chloride, which is treated with the same amount of picric acid and caustic soda (see p. 528).

The Biological Relationship of Creatine and Creatinine.

From the chemical point of view the presence of creatinine in urine would be explained by its formation from the creatine in muscle. The physiological experiments do not bear out this relationship. The daily amount of creatinine in urine is constant in amount and is derived mainly from the tissues, but very small amounts come also from the food. The addition of creatine to the food does not increase the amount of creatinine in urine. If the amount of creatine eaten be 1 gm., it is not excreted as such, or as creatinine ; if more than 1 gm. be eaten, the excess over 1 gm. is excreted as creatine. The amount of creatinine eliminated is also related to the muscular condition ; less is eliminated at rest, more at work.

Folin has suggested the following explanation :—

Creatine is a normal constituent of the living muscle. Normally, the muscle substance during its life processes gives rise to creatinine. During fasting, in fevers, etc., the normal breakdown is accompanied by the breakdown into creatine. Creatine, taken as food, is absorbed into the muscle and the excess is eliminated. The presence of traces of creatine in urine, or of creatinine in muscle, arise chemically by the action of acids, or alkalies.

CHAPTER XXXII.

HETEROCYCLIC COMPOUNDS.

NUMEROUS compounds exist containing rings, or nuclei, composed of carbon atoms and atoms of other elements, especially oxygen, sulphur and nitrogen. These ring compounds are grouped together as the heterocyclic compounds.

Some of the heterocyclic compounds are closely connected with the aliphatic series of compounds, e.g. the anhydrides of dibasic acids, such as succinic anhydride; the γ-lactones and other lactones; the imides from ammonium salts of dibasic acids, such as succinimide; the polymers of the aldehydes, such as trioxymethylene, paraldehyde; and of cyanic acid, namely, cyanuric acid. Creatinine may also be placed in this group. In these compounds the ring is formed easily and it is easily ruptured. They are therefore usually considered as aliphatic compounds. Other heterocyclic compounds possess a more stable ring and they resemble the aromatic substances very closely in their properties. They include pyridine, quinoline and their derivatives, the alkaloids.

Intermediately between these two classes there are other heterocyclic ring compounds which do not possess the chief properties of aromatic compounds in forming nitro- and sulphonic acid derivatives, but they possess a ring which is comparatively stable and is not easily broken down. In this group are included the cyclic ureides, pyrimidine and purine and their derivatives, pyrrole, thiophene, furfurane.

As in the carbocyclic compounds the rings containing 5 atoms and 6 atoms are the most stable.

A. **UREIDES OF MONOBASIC ACIDS**, e.g. acetyl urea.

In the same way as ammonia forms amides with acids, so also does urea form ureides. Ureides are therefore derivatives of urea with acid radicles.

They are obtained by the action of acid chlorides, or acid anhydrides, upon urea :—

$$CH_3 . CO . Cl + H_2N . CO . NH_2 = HCl + CH_3 . CO—NH . CO . NH_2.$$

They are solid compounds. Acetyl urea forms long silky needles which melt at 214° and which are not easily soluble in cold water, or alcohol. They are neutral in reaction and do not form salts with acids.

Like amides, they are easily decomposed by hydrolysis, especially by alkalies, and are converted into their constituents :—

$$CH_3 . CO—NH . CO . NH_2 + H_2O = CH_3 . COOH + H_2N . CO . NH_2.$$
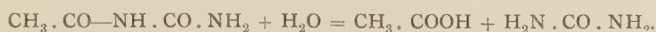
The urea may be decomposed into ammonia and carbon dioxide.

Diacetyl urea, $CH_3 . CO—NH . CO . NH—CO . CH_3$, is formed by the action of carbonyl chloride upon acetamide.

B. UREIDES OF DIBASIC ACIDS.

Two classes of ureides are formed with these acids :—

(1) open chain compounds, such as oxaluric acid.

(2) closed chain compounds, such as oxalylurea, or parabanic acid.

Oxaluric Acid.   Oxalylurea, or Parabanic Acid.
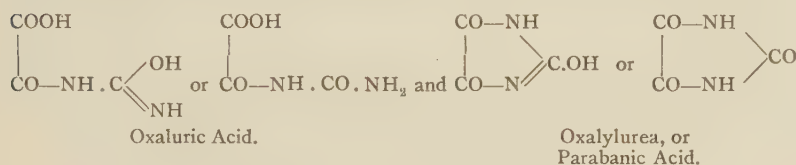
The open chain compounds have a free —COOH group, and are termed -uric acids, e.g. also malonuric acid.

The closed chain compounds are termed Cyclic Ureides (below).

Oxaluric acid is obtained by the action of bromine upon parabanic acid, and by the action of alkalies upon salts of parabanic acid.

Oxaluric acid is a crystalline powder soluble in water with difficulty and is present in small quantities in urine.

Both parabanic acid and oxaluric acid are decomposed by hydrolysis by boiling with water, acids, or alkalies into urea and oxalic acid.

C. UREIDO ACIDS.

Ureides of hydroxy and aldehyde acids do not exist. On the other hand, combinations of urea with the hydroxyl group, or aldehyde group, of such acids are known. They correspond to the amino acids and are ureido acids.

Hydantoic acid and **allantoic acid** are derived from glycollic and glyoxylic acids :—

Hydantoic acid, or Ureido-acetic acid.   Allantoic acid, or Di-ureido-acetic acid.
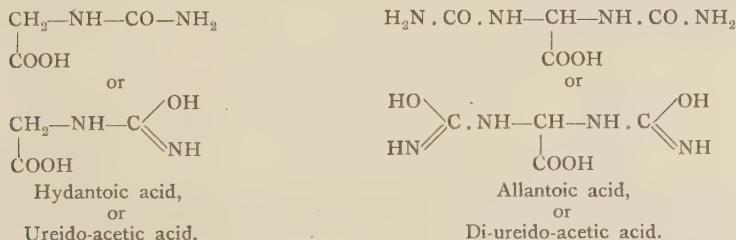
The simple ureido acids may be obtained by boiling the hydroxy acid with urea. They can be synthesised from the amino acid by the action of potassium cyanate :—

$$\begin{array}{c} CH_2.NH_2 \\ | \\ COOH \end{array} + HOCN \rightleftarrows HNCO = \begin{array}{c} CH_2.NH-C{\Large\langle}^{OH}_{NH} \\ | \\ COOH. \end{array}$$
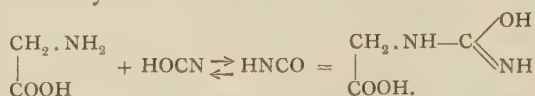
Hydantoic acid was first obtained from hydantoin by boiling it with baryta water :—

$$\begin{array}{c} CH_2-HN{\Large\diagdown} \\ {\Large\diagup}CO + H_2O = \\ CO-HN \end{array} \begin{array}{c} CH_2-NH-CO-NH_2 \\ | \\ COOH. \end{array}$$
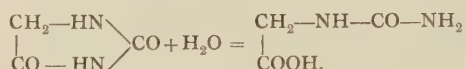
Hydantoic acid is a white solid, easily soluble in water and alcohol. It is converted into glycine, carbon dioxide and ammonia on heating with hydriodic acid.

Hydantoic acid is converted by boiling with dilute mineral acid, or by cold concentrated hydrochloric acid, into hydantoin :—

$$\begin{array}{c} CH_2-NH-CO-NH_2 \\ | \\ COOH \end{array} = H_2O = \begin{array}{c} CH_2-NH{\Large\diagdown} \\ | {\Large\diagup}CO \\ CO-NH \end{array}$$
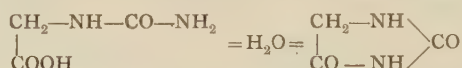
Allantoic acid is obtained from allantoin by the action of cold caustic potash :—

$$\begin{array}{c} H_2N.CO.NH.CH.NH{\Large\diagdown} \\ | {\Large\diagup}CO + H_2O = \\ CO-NH \end{array} \begin{array}{c} H_2N.CO.NH.CH.NH.CO.NH_2 \\ | \\ COOH. \end{array}$$
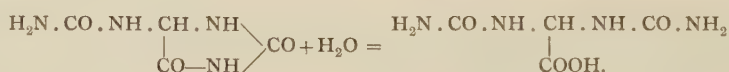
Allantoic acid is slightly soluble in water, but easily soluble in alkalies. It is converted, on boiling with water, into glyoxylic acid and urea.

The hydroxy acids and aldehyde acids form cyclic ureides by combination of both the acid and alcohol, or aldehyde, group with urea.

D. CYCLIC UREIDES.

The cyclic ureides are formed by combination of hydroxy acids and dibasic acids with urea. The first series comprises hydantoin, allantoin, and parabanic acid :—

$$\begin{array}{c} CH_2-NH{\Large\diagdown} \\ | {\Large\diagup}CO \\ CO-NH \end{array} \qquad \begin{array}{c} H_2N.CO.NH.CH-NH{\Large\diagdown} \\ | {\Large\diagup}CO \\ CO-NH \end{array} \qquad \begin{array}{c} CO-NH{\Large\diagdown} \\ | {\Large\diagup}CO \\ CO-NH \end{array}$$
$$\text{Hydantoin.} \qquad\qquad\qquad \text{Allantoin.} \qquad\qquad\qquad \text{Parabanic acid.}$$
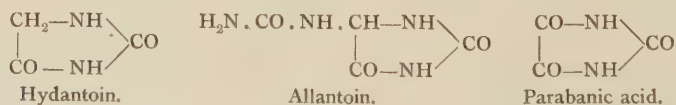
These compounds contain rings with 5 atoms, and are often grouped with the glyoxaline compounds (below).

The second series of cyclic ureides is derived from dibasic acids containing three carbon atoms in their molecule :—

$$
\begin{array}{llll}
\text{CO——NH} & \text{CO———NH} & \text{CO——NH} & \text{CO——NH} \\
\quad|\qquad| & \quad|\qquad| & \quad|\qquad| & \quad|\qquad| \\
\text{CH}_2\quad\text{CO} & \text{CHOH}\quad\text{CO} & \text{C(OH)}_2\ \text{CO}\ \ \text{or} & \text{CO}\quad\text{CO} \\
\quad|\qquad| & \quad|\qquad| & \quad|\qquad| & \quad|\qquad| \\
\text{CO——NH} & \text{CO———NH} & \text{CO——NH} & \text{CO——NH}
\end{array}
$$

Malonyl urea,	Tartronyl urea,	Mesoxalyl urea,	
or	or	or	
barbituric acid.	dialuric acid.	alloxan.	

$$
\begin{array}{lll}
\text{CO———NH} & \text{CO———NH} & \text{CO———NH} \\
\quad|\qquad| & \quad|\qquad| & \quad|\qquad| \\
\text{CH.NO}_2\ \text{CO} & \text{CH.NH}_2\ \text{CO} & \text{C:NOH}\ \text{CO} \\
\quad|\qquad| & \quad|\qquad| & \quad|\qquad| \\
\text{CO———NH} & \text{CO———NH} & \text{CO———NH}
\end{array}
$$

Nitromalonyl urea,	Aminomalonyl urea,	Isonitrosomalonyl urea,
or	or	or
dilituric acid.	uramil.	violuric acid.

$$
\begin{array}{ll}
\text{CO———NH} & \text{CO————————NH} \\
\quad|\qquad| & \quad|\qquad| \\
\text{C(C}_2\text{H}_5)_2\ \text{CO} & \text{CH.NHC(OH)NH}\ \text{CO} \\
\quad|\qquad| & \quad|\qquad| \\
\text{CO————NH} & \text{CO————————NH}
\end{array}
$$

Diethylbarbituric acid,	Carbamidomalonyl urea,
or	or
veronal.	pseudo-uric acid.

Some of these ureides were first obtained by the oxidation of uric acid and were the fundamental substances from which its constitution was determined. Others were prepared synthetically in the study of these ureides and in the synthesis of uric acid.

This series of compounds contains rings with 6 atoms, and belongs to the pyrimidine compounds (below).

Both series of cyclic ureides have distinct differences from glyoxaline and pyrimidine compounds, and are conveniently kept in a separate group.

All the cyclic ureides have acid properties and form salts. This property is due to the presence of the—CO—NH—CO grouping which makes the hydrogen attached to nitrogen replaceable by metals.

Hydantoin.

Hydantoin was first obtained by heating allantoin with hydriodic acid. It is prepared synthetically by heating bromacetyl urea with alcoholic ammonia :—

$$
\begin{array}{ccc}
\text{NH.OC.CH}_2\text{Br} & & \text{NH—OC} \\
\diagup & & \diagup\quad\ | \\
\text{CO} & = & \text{CO}\qquad | \quad + \text{HBr.} \\
\diagdown & & \diagdown\quad\ | \\
\text{NH}_2 & & \text{NH——CH}_2
\end{array}
$$
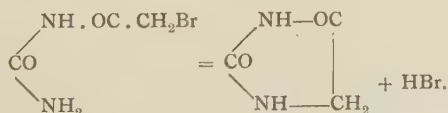
It is a white crystalline solid which melts at 216°.

Allantoin.

Allantoin, a compound of glyoxylic acid with 2 molecules of urea, is derived from the hypothetical dihydroxy-acetic acid.

Allantoin was first found in the allantoic fluid of calves; it has since been found in the urine of various animals, ox, dog, monkey, rabbit, sheep, and also in various organs of animals. It has been found in plants. Allantoin is an oxidation product of uric acid with permanganate, and arises in those animals in which allantoin is present in the urine by the oxidation of uric acid.

Preparation.

100 gm. of uric acid are suspended in 1500-2000 c.c. of water and dissolved by the careful addition of caustic soda in small quantities at a time. The alkaline liquid is treated with a concentrated solution of 62 gm. of potassium permanganate and well stirred. Manganese dioxide is formed and the permanganate decolorised. This takes place rapidly and is complete in about an hour; it must be tested for by filtering a sample, if necessary. As soon as the solution is decolorised, it is filtered from manganese dioxide, acidified with acetic acid and evaporated (best *in vacuo*) until it crystallises. The crystals are recrystallised from hot water.

Properties.

Allantoin forms shining colourless prisms which have no taste, or smell, and are neutral in reaction to litmus. It is not easily soluble in cold water (1 in 160 parts), or cold alcohol, but easily soluble in hot water and hot alcohol; on heating, it turns brown at 220° and melts with decomposition at 231°.

Allantoin forms compounds with metals : it is precipitated by ammoniacal silver solutions : the precipitate is soluble in ammonia ; it is also precipitated by salts of lead, copper, and mercury (see above). It reduces Fehling's solution on prolonged boiling.

Allantoin is decomposed by hydrolysis with acids, or alkalies, giving urea (or ammonia and carbon dioxide) and acetic acid and oxalic acid. It is also decomposed by hypobromite solution with evolution of nitrogen.

Parabanic acid, or Oxalylurea, is formed on oxidising uric acid with nitric acid. It is prepared synthetically by the action of phosphorus oxychloride upon a mixture of urea and oxalic acid.

Parabanic acid is a white crystalline substance, which is soluble in water and alcohol.

Alloxan.

Alloxan is the most interesting of the series of compounds derived from acids with 3 carbon atoms as it formed the central point in our knowledge of the constitution of uric acid and other purines (p. 315).

Alloxan is produced by the careful oxidation of uric acid with nitric acid, bromine, or chlorine ; also by the oxidation of xanthine.

Preparation.

It is easier to prepare alloxantin from uric acid and then to prepare alloxan from alloxantin.

Alloxantin may be prepared as follows : 10 gm. of uric acid are covered with 20 c.c. of water and 20 gm. of concentrated hydrochloric acid and heated to 35° ; 2·5 gm. of powdered potassium chlorate are gradually added with continuous stirring. The uric acid dissolves and a pale yellow liquid results. This is diluted with about 75 c.c. of water, allowed to stand and filtered. The filtrate, which contains alloxan, is saturated with hydrogen sulphide and allowed to stand for 12-16 hours. Alloxantin mixed with sulphur separates out ; it is filtered off and washed with water. It is separated from sulphur by solution in a small quantity of boiling water from which it separates on cooling in colourless crystals. These crystals are sometimes tinged with pink.

Alloxantin is readily reduced, or oxidised.

Alloxan is prepared : 3 gm. of finely powdered alloxantin are mixed with 3 gm. of concentrated nitric acid and 7 gm. fuming nitric acid (sp. gr. 1·5). Slow oxidation occurs on standing and large crystals of alloxan are formed. The oxidation is complete in about 2 days and is shown by the complete solubility of the crystals in water. The crystals are placed on a porous plate to drain off the nitric acid and dried in the air. They are recrystallised from water.

Properties.

Alloxan is a white crystalline substance which separates from water in long shining rhombic prisms containing 4 molecules of water of crystallisation ; on exposure to air, the crystals effloresce and lose 3 molecules of water ; the last molecule of water is driven off by heating to 150°.

It is easily soluble in water ; the solution has an acid reaction and disagreeable taste and it slowly turns the skin a purple-red. A deep indigo blue colour is formed when ferrous sulphate is added to its solution.

If a few drops of its solution in water be evaporated to dryness and the reddish residue be treated with ammonia, it turns purple (murexide).

Reactions.

(1) Alloxantin is formed by the action of reducing agents upon alloxan in the cold :—

Alloxantin.
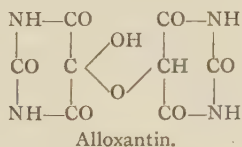
It is formed by combination of alloxan and dialuric acid (p. 309).

(2) Dialuric acid is formed by the action of reducing agents on alloxan on warming.

(3) Parabanic acid and carbon dioxide are formed by the oxidation of alloxan with boiling dilute nitric acid.

(4) Barbituric acid is obtained from alloxantin by the action of concentrated sulphuric acid.

(5) Dilituric acid is formed by the action of fuming nitric acid upon alloxantin, or by the oxidation of violuric acid.

(6) Violuric acid is formed by the action of potassium nitrite upon alloxantin, or by the action of hydroxylamine upon alloxan.

(7) Uramil is formed by the reduction of dilituric acid and violuric acid.

These ureides are white crystalline substances which are easily soluble in water; uramil is only slightly soluble and becomes red on exposure to the air.

E. GLYOXALINE, OR IMINAZOLE, AND DERIVATIVES.

The cyclic ureides, hydantoin, allantoin, and parabanic acid contain a ring made up of 2 nitrogen atoms and 3 carbon atoms.

The ring structure

$$\begin{array}{c} C-N \\ \| \quad \rangle C \\ C-N \end{array}$$

is known as the glyoxaline, or iminazole, ring.

It is present in histidine, histamine, and urocanic acid, three important physiological compounds.

Histidine is β-iminazole-α-aminopropionic acid.

Histamine is β-iminazole-ethylamine.

Urocanic acid is β-iminazole-acrylic acid.
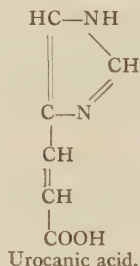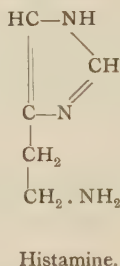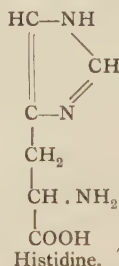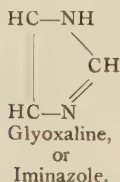
Histidine is a constituent of proteins and is contained in greatest amount in the protein, hæmoglobin.

Histamine is a product of the putrefaction of histidine (or proteins). It is a constituent of ergot and is present in putrified meat, etc. It has a marked physiological action upon the sympathetic nervous system.

Urocanic acid has been isolated from the urine of dogs and also from a trypsin digest of protein.

Pilocarpine.

This basic substance, or alkaloid, contains an iminazole ring. Its formula is probably the following :—

$$C_2H_5 . CH \underset{\underset{CO-O-CH_2}{|}}{\overset{}{\rule{1cm}{0.4pt}}} CH-CH_2-C-N \underset{\underset{H . C-N}{\|}}{\overset{CH_3}{\diagup}} CH.$$
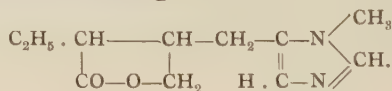
F. PYRIMIDINE AND DERIVATIVES.

The cyclic ureides derived from urea and acids containing three carbon atoms (p. 309) are heterocyclic compounds and belong to the group of pyrimidines. The pyrimidines are the group of compounds with the structure :—

$$
\begin{array}{cc}
1\,N\!\!-\!\!C\,6 \\
|\quad| \\
2\,C\quad C\,5 \\
|\quad\| \\
3\,N\!\!-\!\!C\,4
\end{array}
$$
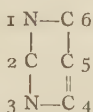
and are really cyclic ureides derived from urea and the unsaturated acids, acrylic acid, methylacrylic acid, crotonic acid.

To this group belong the three compounds, thymine, uracil, and cytosine, which are constituents of nucleic acid :—
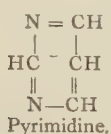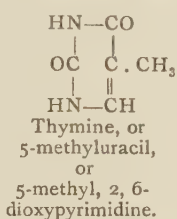
$N = CH$	$HN\!-\!CO$	$N = C\,.\,NH_2$	$HN\!-\!CO$
$HC\;\;\;CH$	$OC\;\;\;C\,.\,CH_3$	$OC\;\;\;CH$	$OC\;\;\;CH$
$N\!-\!CH$	$HN\!-\!CH$	$HN\!-\!CH$	$HN\!-\!CH$
Pyrimidine.	Thymine, or 5-methyluracil, or 5-methyl, 2, 6-dioxypyrimidine.	Cytosine, or 6-amino-2-oxy-pyrimidine.	Uracil, or 2, 6-dioxy-pyrimidine.
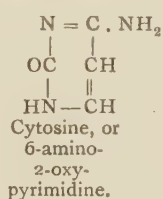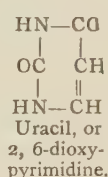
The ring contained in these compounds is a portion of the ring structure of the purines and it was first supposed that cytosine and uracil were decomposition products of adenine and guanine, but it has been definitely proved that these compounds are not secondary products and that they are part of the molecule of nucleic acid (p. 325).

These three compounds have been prepared by synthesis and their constitution established. They are most readily prepared from nucleic acid.

Plant nucleic acid yields uracil and cytosine.

Animal nucleic acid yields thymine and cytosine. The method of preparation is described in W. Jones' monograph on "Nucleic Acids."

CHAPTER XXXIII.

PURINES.

URIC acid, xanthine, hypoxanthine, guanine, adenine, caffeine, theobromine and others are classed together in the special group of compounds known as the purines. Not only are these compounds found associated in nature in both animals and plants, but also they are chemically very closely related. They yield alloxan, or dimethyl alloxan, on oxidation, and have many other similar reactions.

All these compounds have been synthesised by Emil Fischer and their exact chemical relationship to one another established. The result of these investigations has shown that they are all derived from the compound purine, which stands in the same kind of relationship to them as a hydrocarbon does to an alcohol, an amine, etc. Thus :—

Purine		$C_5H_4N_4$
Hypoxanthine	= monoxypurine	$C_5H_4N_4O$
Xanthine	= dioxypurine	$C_5H_4N_4O_2$
Uric acid	= trioxypurine	$C_5H_4N_4O_3$
Adenine	= aminopurine	$C_5H_3N_4 . NH_2$
Guanine	= amino-oxypurine	$C_5H_3N_4 . O . NH_2$
Theobromine	= dimethyl dioxypurine	$C_5H_2N_4O_2(CH_3)_2$
Theophyllin	= dimethyl dioxypurine	$C_5H_2N_4O_2(CH_3)_2$
Caffeine	= trimethyl dioxypurine	$C_5HN_4O_2(CH_3)_3.$

The compounds have the heterocyclic ring structure in which the atoms are numbered in the following order :—

$$
\begin{array}{cc}
{}^1N\!\!-\!\!{}^6C & \\
| \quad | & \\
{}^2C \quad {}^5C\!\!-\!\!{}^7N & \\
| \quad \| & \searrow C^8. \\
{}^3N\!\!-\!\!{}^4C\!\!-\!\!N^9 &
\end{array}
$$
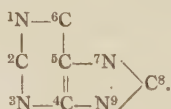
This ring structure is a combination of the pyrimidine and glyoxaline rings.

The formulæ for the various compounds are :—

$$
\begin{array}{ccc}
N=CH & HN-C=O & HN-C=O \\
| \quad | & | \quad | & | \quad | \\
HC \quad C-NH & HC \quad C-NH & O=C \quad C-NH \\
\| \quad \| \; \searrow & \| \quad \| \; \searrow & \| \quad \| \; \searrow \\
\quad\quad CH & \quad\quad CH & \quad\quad CH \\
N-C-N & N-C-N & HN-C-N \\
\text{Purine.} & \text{Hypoxanthine,} & \text{Xanthine,} \\
& \text{or} & \text{or} \\
& \text{6-monoxypurine.} & \text{2, 6-dioxypurine.}
\end{array}
$$

311

HN—C=O
  | |
O= C C—NH
         \
            C=O
         /
HN—C—NH

Uric acid,
or
2, 6, 8-trioxypurine.

N=C—NH$_2$
HC C—NH
        \
          CH
         /
N—C—N

Adenine,
or
6-aminopurine.

CH$_3$.N—C=O
O=C C—NH
         \
          CH
         /
CH$_3$.N—C—N

Theophylline,
or
1, 3-dimethylxanthine.

HN—C=O
O=C C—N.CH$_3$
        \
         CH
        /
CH$_3$.N—C—N

Theobromine,
or
3, 7-dimethylxanthine.

HN—C=O
H$_2$N—C C—NH
          \
           CH
         /
HN—C—N

Guanine,
or
2-amino, 6-oxypurine.

CH$_3$.N—C=O
O=C C—N.CH$_3$
        \
         CH
       /
CH$_3$.N—C—N

Caffeine,
or
1, 3, 7-trimethylxanthine.

It will be noticed that the complex, or ring, of atoms is the same in all these compounds, but that, according as the compounds contain oxygen atoms attached to a carbon atom, the double bond is changed in position, so as to keep the nitrogen and carbon atoms trivalent and tetravalent respectively. The double bond between the carbon atoms 4 and 5 is the same throughout.

URIC ACID.

Scheele discovered uric acid in urinary calculi in 1776 and also isolated it from urine. He made a careful investigation of its properties and reactions, most of which are still used at the present time for its identification. It was called lithic acid, or ouric acid, by Fourcroy in 1793, who showed that it contained urea. It was discovered in guano in 1805 and in bird's excrement in 1815, and later it was shown to be the chief constituent of snakes' excrement. Prout about this time showed that uric acid, on oxidation with nitric acid, gave alloxan. It was first analysed in 1834 by Liebig and Wöhler and found to possess the empirical formula $C_5H_4N_4O_3$. These workers by oxidising uric acid with lead peroxide obtained allantoin, which had been previously found in the allantoic fluid of calves.

The formation of alloxan and urea, and of allantoin, by the oxidation of uric acid, showed that uric acid contained 2 molecules of urea and that it contained the structures of alloxan and allantoin in its molecule :—

$$
\begin{array}{cc}
\text{N—C} \\
|\ \ | \\
\text{C}\ \ \text{C} \\
|\ \ | \\
\text{N—C}
\end{array}
\qquad
\begin{array}{c}
\text{C—N} \\
|\qquad\diagdown \\
\qquad\quad\text{C.} \\
|\qquad\diagup \\
\text{C—N}
\end{array}
$$
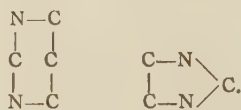
Two formulæ were put forward to represent the constitution of uric acid :—

$$
\begin{array}{c}
\text{HN—CO} \\
|\qquad| \\
\text{OC}\quad \text{C—NH} \\
|\qquad\|\qquad\diagdown \\
\qquad\qquad\qquad\text{CO} \\
|\qquad\|\qquad\diagup \\
\text{HN—C—NH} \\
\text{Formula} \\
\text{of} \\
\text{Medicus.}
\end{array}
\qquad\qquad
\begin{array}{c}
\text{HN—C——NH} \\
|\quad\ \diagdown\quad| \\
\text{OC}\quad|\quad\text{CO CO} \\
|\quad\diagup\quad| \\
\text{NH—C—·—NH} \\
\text{Formula} \\
\text{of} \\
\text{Fittig.}
\end{array}
$$
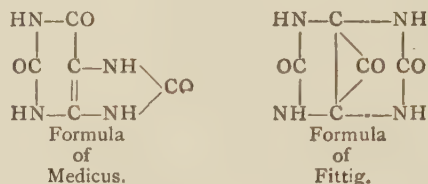
The formula proposed by Medicus was ultimately proved to be the correct one by the synthesis of uric acid by Fischer.

The previous syntheses of uric acid by Horbaczewski (1) by fusing together glycocoll and urea, (2) by combining trichlorlactamide with urea :—

$$
\begin{array}{c}
\text{NH}_2 \\
| \\
\text{CO} \\
| \\
\text{NH}_2
\end{array}
+ .
\begin{array}{c}
\text{CCl}_3 \\
| \\
\text{CHOH} \\
| \\
\text{CO . NH}_2
\end{array}
+
\begin{array}{c}
\text{H}_2\text{N} \\
| \\
\text{CO} \\
| \\
\text{H}_2\text{N}
\end{array}
=
\begin{array}{c}
\text{HN—C—HN} \\
|\quad\|\qquad\diagdown \\
\text{OC}\quad\text{C—HN}\qquad\text{CO} \\
|\quad|\qquad\diagup \\
\text{HN—CO}
\end{array}
$$
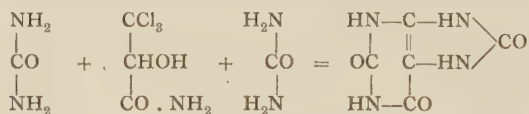
did not definitely prove its constitution. The synthesis by Behrend and Roosen from aceto-acetic acid and the following synthesis commenced by Baeyer and completed by Fischer proves the constitution of uric acid :—

(1) Malonyl urea, or barbituric acid, is obtained by heating urea and malonic acid with phosphorus oxychloride.

(2) Nitrous acid converts malonyl urea into oximidomesoxalyl urea, or violuric acid.

(3) Aminomalonyl urea, or uramil, is obtained by reducing violuric acid.

(4) Potassium cyanate converts uramil by rearrangement, as in the formation of urea, into pseudo-uric acid.

(5) Pseudo-uric acid loses water on heating with fused oxalic acid, or boiling with hydrochloric acid, and is changed into uric acid.

$$
\begin{array}{c}
\text{COOH} \\
| \\
\text{CH}_2 \\
| \\
\text{COOH} \\
\text{Malonic} \\
\text{acid.}
\end{array}
\rightarrow
\begin{array}{c}
\text{NH—CO} \\
|\qquad| \\
\text{CO}\quad\text{CH}_2 \\
|\qquad| \\
\text{NH—CO} \\
\text{Barbituric} \\
\text{acid.}
\end{array}
\rightarrow
\begin{array}{c}
\text{NH—CO} \\
|\qquad| \\
\text{CO}\quad\text{C=NOH} \\
|\qquad| \\
\text{NH—CO} \\
\text{Violuric} \\
\text{acid.}
\end{array}
\rightarrow
\begin{array}{c}
\text{NH—CO} \\
|\qquad| \\
\text{CO}\quad\text{CH . NH}_2\rightarrow \\
|\qquad| \\
\text{NH—CO} \\
\text{Uramil.}
\end{array}
$$

$$
\begin{array}{c}
\text{NH—CO} \\
|\qquad| \\
\text{CO}\quad\text{CH . NH}_2 \\
|\qquad| \\
\text{NH—CO}
\end{array}
\text{. HOCN} \rightleftarrows \text{HNCO}
\begin{array}{c}
\text{NH—CO} \\
|\qquad| \\
\text{CO}\quad\text{CH . NH . C(OH)NH} \rightarrow \\
|\qquad| \\
\text{NH—CO} \\
\text{Pseudo-uric} \\
\text{acid.}
\end{array}
\begin{array}{c}
\text{NH—CO} \\
|\qquad| \\
\text{CO}\quad\text{C—NH} \\
|\qquad\|\qquad\diagdown\text{CO.} \\
\text{NH—C—NH}\diagup \\
\text{Uric} \\
\text{acid.}
\end{array}
$$
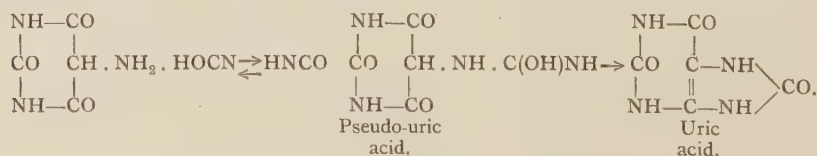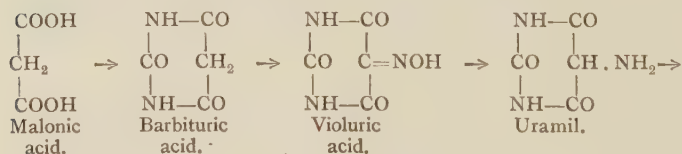
Preparation.

* (1) *From Snakes' Excrement, or Guano.*

·5 to 1 gm. of snakes' excrement (or guano) is powdered, suspended in 100 c.c. of water, heated nearly to boiling and dissolved by adding dilute sodium carbonate. The solution is heated until ammonia is no longer evolved and filtered from insoluble material (sand, etc.). Excess of dilute hydrochloric acid is added to the filtrate. Uric acid is precipitated; it is filtered off when the solution has cooled and washed free from acid with water. The product is generally almost pure, but may be purified by dissolving in sodium carbonate solution and reprecipitating with acid. It is dried in the air, or at 100°.

(2) *From Human Urine.*

* A twenty-four hours' quantity of human urine contains from 0·5-1·5 gm. of uric acid, sometimes as much as 2·0 or 2·5 gm.

(*a*) 500 c.c. of urine are treated with 50 c.c. of concentrated hydrochloric acid and allowed to stand in a cool place for twenty-four hours. Pigmented crystals of uric acid slowly separate out and adhere to the sides of the vessel. Microscopic examination of the crystals shows that they consist of irregular, much pigmented crystals, generally arranged in sheaves (Fig. 42, p. 318, Fig. 62, p. 532).

* (*b*) 100 c.c. of urine are saturated with crystals of ammonium chloride (27 gm. necessary) and 1 or 2 drops of strong ammonia are added. A gelatinous precipitate of ammonium hydrogen urate is formed. This is filtered off after about fifteen minutes. It can be shown to contain uric acid by testing a small portion by the murexide test. The uric acid is obtained from the precipitate by dissolving it in the smallest quantity of hot water containing a drop of sodium hydroxide, filtering, if necessary, and acidifying with a drop of concentrated hydrochloric acid. Uric acid crystallises out on cooling, if too much water has not been used in dissolving the ammonium urate.

Properties.

Pure uric acid is a colourless crystalline powder, but as obtained from solutions containing urinary pigments it is generally more or less pigmented; the pigment is difficult to remove by treatment with animal charcoal. The crystals usually consist of rhombic plates, or prisms, but various shapes are observed depending on the rate of its crystallisation from solution. These are shown in Fig. 42.

Uric acid has no taste or smell. It is only slightly soluble in water —1 part in 39,500 parts of water at 18°, 1 part in about 1900 parts of

hot water.   It is insoluble in alcohol and ether, but soluble in glycerol.
It is soluble in solutions of the borates, phosphates, carbonates, and
acetates of the alkali metals, with the formation of acid salts of these
acids and of uric acid.   It dissolves in concentrated sulphuric acid from
which it is precipitated by the addition of water.

Uric acid is a weak acid and forms two series of salts, neutral and
acid salts.   The formation of salts can be explained either by the
tautomeric formula in which hydroxyl groups attached to the carbon

$$
\begin{array}{c}
N=\!C \cdot OH \\
\mid \quad \mid \\
HO \cdot C \quad C—NH \\
\parallel \quad \parallel \quad \diagdown C \cdot OH \\
N—C—N
\end{array}
$$
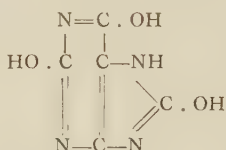
atoms are present, evidence of which is shown by the action of phos-

Fig. 42.—Uric acid.   (After Funke.)

phorus trichloride upon uric acid,
which gives trichloropurine, or to
the presence of —CO . NH—CO
groups which influence the pro-
perties of the hydrogen atoms at-
tached to the N atoms so that they
are acidic in character and replace-
able by metals (see cyclic ureides).

The neutral salts, such as
$C_5H_2Na_2N_4O_3$, are comparatively
easily soluble in water and are
obtained on dissolving uric acid in
alkali hydroxides, or in hot solu-
tions of the carbonates.   Lithium
urate is the most soluble salt of uric acid.

Uric acid is insoluble in cold solutions of the carbonates and may
thus be separated from other acids, such as benzoic acid.

The acid salts, such as $C_5H_3NaN_4O_3$, are soluble with difficulty in
water.   They are obtained on passing carbon dioxide into a solution
of the neutral salt.

One part of acid sodium urate is soluble in 1100-1200 parts of cold
water and 125 parts of hot water; 1 part of acid potassium urate is
soluble in 800 parts of cold water and in 70 parts of hot water;
1 part of acid ammonium urate is soluble in 1600 parts of cold water,
more easily in hot water and is insoluble in ammonium chloride solution.

A double compound of uric acid and acid sodium urate, $C_5H_4N_4O_3$
+ $C_5H_3NaN_4O_5$, is said to be deposited in gouty joints and cartilages.

All the salts are decomposed by acetic acid, or hydrochloric acid, with the gradual separation of uric acid.

FIG. 43.—Sodium urate.
(After Funke.)
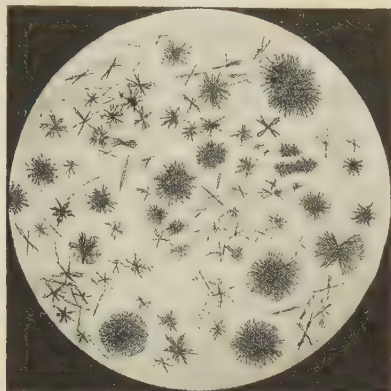
FIG. 44.—Ammonium urate.
(After Funke.)

Reactions.

* (1) On heating, uric acid is decomposed with the formation of urea, ammonium carbonate, cyanuric acid and hydrogen cyanide and a charred mass remains.

* (2) Uric acid is decomposed on heating with solid potassium hydroxide with the formation of ammonia and potassium cyanide; the presence of potassium cyanide may be shown by extracting the residue with water and testing for cyanides (p. 117).

* (3) Uric acid chars on heating with concentrated sulphuric acid.

* (4) *Murexide Test.*—On evaporating a small quantity of uric acid, or an urate, to dryness with dilute nitric acid on a crucible lid, a yellow, or yellowish-red, residue is left. On adding to it a drop of ammonia with a glass rod, the colour changes to purple; ammonium purpurate, or murexide, is formed. A drop of caustic soda gives a blue-violet colour.

Alloxantin (p. 311) is formed in this reaction by the action of dilute nitric acid. Its ammonium salt is murexide :—

$$
\begin{array}{cccc}
\text{NH—CO} & & \text{CO—NH} & \\
|\quad\ | & \diagup\text{ONH}_4\ \ | \quad | & \\
\text{CO}\quad \text{C} & < \qquad \text{CH}\quad \text{CO} & \\
|\quad\ | & \diagdown\ \text{O}\ \diagup\ |\quad\ | & \\
\text{NH—CO} & & \text{CO—NH.} &
\end{array}
$$
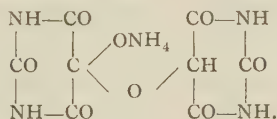
* (5) *Schiff's Test.*—A solution of uric acid in sodium carbonate solution reduces silver nitrate. This is best observed by pouring some of the urate solution upon a filter paper moistened with silver nitrate. A black stain of metallic silver results.

*   (6) *Fehling's Solution.*—A white precipitate of copper urate is formed when a solution of uric acid is added to Fehling's solution and warmed. On boiling for some time, the solution is reduced with the formation of cuprous oxide.

Note.—On this account urine on prolonged boiling reduces Fehling's solution and the reduction may be wrongly attributed to small amounts of glucose (see under pathological urines).

(7) If a small quantity of uric acid be carefully heated with dilute nitric acid just to effervescence and the excess of acid be carefully evaporated so as to avoid coloration, a blue colour results on the addition of 2-3 drops of concentrated sulphuric acid and a few drops of commercial benzene (containing thiophene). The colour changes to brown on evaporation of the benzene, but returns on again adding benzene (*Denigès*).

(8) Dilute solutions of uric acid are completely precipitated by the addition of ammoniacal silver nitrate and magnesia mixture. Silver magnesium urate is formed.

(9) Dilute solutions of uric acid are precipitated by adding copper sulphate and sodium bisulphite. On boiling, cuprous urate is formed.

These two reactions (8) and (9) are used in the precipitation of uric acid and the other purines from urine and extracts of tissues.

(10) Very dilute solutions of uric acid containing ·5 mg. give an intense blue colour with a specially prepared phosphotungstic acid reagent (*Folin*).

Estimation of Uric Acid.

*   (1) Uric acid is readily oxidised by potassium permanganate and converted into allantoin and other products of its oxidation. Use is made of this reaction for its estimation. From solutions, such as urine, in which other organic substances are present, the uric acid must be precipitated before it can be estimated (p. 526).

·05N potassium permanganate (1·581 gm. per litre) is commonly used in the process.

The permanganate solution is placed in a burette with a glass tap (rubber cannot be used as it is attacked by permanganate). The level of the liquid in the burette is most conveniently read by holding a lighted match behind it.

100 c.c. of a solution of uric acid, or the same volume of a solution containing a weighed quantity of uric acid, or solid urate (·5-1 gm. dissolved in water containing soda and diluted to 1 litre), are placed in a flask, or beaker, 20 c.c. of concentrated sulphuric acid are added, the solutions are well mixed and the permanganate is run in whilst the solution is hot.

At first every drop of permanganate is decolorised before it diffuses through the liquid. The end point is reached as soon as a drop produces a pink flush throughout the liquid. This pink colour

disappears on standing; another drop of permanganate will again produce a pink flush. This can be continued for some time so that the first pink flush must be carefully looked for.

Calculation :—

    1 c.c. of ·05N $KMnO_4$ corresponds to 0·00375 gm. uric acid.

    ∴ x c.c. correspond to x × ·00375 gm in 100 c.c. solution.

(2) Folin has shown that quantities of uric acid amounting to 1 mg. in 1 c.c. can be accurately estimated by means of the colour reaction with phosphotungstic acid. This method is particularly useful for estimating uric acid in blood and can be used for estimating uric acid in small quantities of urine.

Xanthine.

Xanthine was discovered in an urinary calculus by Marcet in 1817, and in 1859 Scherer found it in meat and pancreas. It has since been shown to be present in other animal organs and to be widely distributed in plants.

Its constitution was indicated by its products of oxidation, alloxan and urea; Fischer proved it by synthesis.

Xanthine is most easily prepared by the action of nitrous acid upon guanine. Its preparation from extracts of tissues involves a complicated process of separation from other purines.

Xanthine is a colourless powder which assumes a waxy appearance on rubbing. It separates slowly from its solution in alkali on the addition of acetic acid in the form of colourless nodules consisting of microscopic rhombic shining platelets containing 1 molecule of water of crystallisation.

It is very slightly soluble in water—1 part in 14,151 parts at 16°, 1 part in 1300-1500 parts at 100°. It is insoluble in alcohol and ether. It is easily soluble in caustic alkalies and in 2 per cent. ammonia. On evaporation of the ammoniacal solution, xanthine separates in groups of platelets.

Ammoniacal silver nitrate precipitates it from solution as $C_5H_4N_4O_2$. Ag_2O; the precipitate is soluble in nitric acid and from this solution xanthine silver nitrate, $C_5H_4N_4O_2$. $AgNO_3$, slowly separates in aggregates of tiny needles. This compound is soluble with difficulty in nitric acid. It is precipitated by copper sulphate and sodium bisulphite.

Reactions.

(1) On evaporation with nitric acid, xanthine leaves a yellow residue, which is coloured red by caustic soda and becomes purple on heating.

(2) On boiling a small quantity of xanthine with chlorine water, or with dilute hydrochloric acid and a small crystal of potassium chlorate, and evaporating the solution to dryness, a white, or pale yellow, residue is left. On bringing this into contact with ammonia vapour under a glass cover, it changes to a rose-red colour (murexide).

Hypoxanthine.

Hypoxanthine, like xanthine, occurs widely distributed in the tissues of animals and plants. Its constitution has been proved by synthesis by Emil Fischer.

21

It is prepared most easily by the action of nitrous acid upon adenine. Its isolation from tissues necessitates a complicated process of separation from other purine bases.

Hypoxanthine forms colourless microscopic crystals, soluble with difficulty in water; 1 part is soluble in 1400 parts of water at 19° and in 70 parts of boiling water. It is practically insoluble in alcohol.

It is soluble in dilute acids and alkalies and in ammonia and forms salts with acids, bases and other salts, which crystallise readily. The nitrate $C_5H_4N_4O . HNO_3 + H_2O$ is insoluble in nitric acid.

It is precipitated from solution by ammoniacal silver nitrate and by copper sulphate and sodium bisulphite.

Hypoxanthine differs from xanthine in not giving reactions with nitric acid and chlorine water.

Guanine.

Guanine is also found widely distributed in the tissues of animals and plants and is a constituent of nucleic acid. It is the chief constituent of the excrement of spiders and is found in Peru guano in small quantities. It is deposited in the muscles and joints of pigs in certain cases of illness and it occurs in fish scales and other epidermal structures of fishes.

Fischer has proved its constitution by synthesis.

Guanine forms a colourless, generally amorphous, powder. It is insoluble in water, alcohol, ether and soluble with difficulty in ammonia. It is easily soluble in all mineral acids and alkalies.

Guanine forms a very insoluble picrate, $C_5H_5N_5O . C_6H_3N_3O_7 + H_2O$. The picrates of xanthine and hypoxanthine are more soluble.

Its compound with silver nitrate $C_5H_5N_5O . AgNO_3$ is almost insoluble in cold nitric acid, but more soluble in hot, from which it crystallises on cooling.

Guanine, on evaporation with dilute nitric acid, leaves a brownish-red residue, which becomes bluish-violet on heating.

Adenine.

Adenine was first obtained by Kossel from the pancreas, but has since been obtained from other organs and from plants. Its constitution was proved by synthesis (Emil Fischer).

Adenine forms long colourless needles with 3 molecules of water of crystallisation, or whetstone-like crystals; the former become opaque on exposure to air and if heated in insufficient water become opaque at 53°. It sublimes at 220° and at 250° partially decomposes.

It is soluble with difficulty in cold water, 1 part in 1086 parts, but it is more easily soluble in hot water. The solution has a neutral reaction. It dissolves in alkalies, mineral acids and acetic acid and is precipitated on neutralising the solutions. It is more easily soluble in ammonia than guanine, but less so than hypoxanthine.

Adenine gives no reaction on evaporation with nitric acid. It behaves in a characteristic manner on heating with zinc and hydrochloric acid on the water-bath. The solution turns purple-red; if filtered, made strongly alkaline

with caustic soda and allowed to stand, or shaken with air, it turns ruby-red and then brownish-red. Guanine does not give this reaction, but hypoxanthine gives the colours, though fainter.

Caffeine. Theophylline. Theobromine.

These three compounds are not found in animals, but are fairly widely distributed in plants. Caffeine is the active constituent of tea and coffee. Theobromine is present in cocoa. They produce a stimulating effect on the central nervous system and act as powerful diuretics.

Caffeine is present to the extent of ·8-1·7 per cent. in coffee beans, ·1-·8 per cent. in cocoa beans, 1-2 per cent. in kola nuts, 2-5 per cent. in tea leaves; 2·5-5 per cent. is present in guarana, the roasted fruit of *Paullinia* which is eaten in South America.

Caffeine forms long silky needles containing one molecule of water of crystallisation which it loses at 100°. It melts at 233° and has a bitter taste. It forms salts with mineral salts which are decomposed by water.

Caffeine, on evaporation with chlorine water, leaves a reddish-brown residue which becomes purple when treated with ammonia.

Theobromine is present to the extent of 1·5-2·4 per cent. in cocoa beans; smaller amounts are present in kola nuts and tea leaves; it is not present in coffee beans.

Theobromine forms a crystalline powder which has a bitter taste, is soluble with difficulty in hot water and alcohol, but is easily soluble in ammonia. It forms salts with mineral acids, which are decomposed by water, and with silver nitrate and other metallic salts.

Theophylline was discovered by Kossel in 1888 in extracts of tea and has been synthesised by Emil Fischer.

It forms a white powder which melts at 264°.

Paraxanthine, or 1, 7-dimethylxanthine
Heteroxanthine, or 7-methylxanthine
1-methylxanthine
Epiguanine, or 7-methylguanine

have been isolated from human urine. They are products formed from caffeine in the organism.

The Biological Relationship of the Purines.

Adenine and guanine are constituents of the nucleic acid of animals and plants.

Whilst still in combination in the molecule of nucleic acid they may be acted upon by enzymes in the tissues and converted respectively into hypoxanthine and xanthine. The nucleic acid will thus contain hypoxanthine and xanthine. Unchanged nucleic acid on decomposition in the tissues will yield adenine and guanine, and changed nucleic acid will give hypoxanthine and xanthine.

Adenine and guanine are acted upon by the enzymes, adenase and guanase, in the tissues and converted into hypoxanthine and xanthine.

21 *

These enzymes are present in most organs, but not in all organs; some-times an organ contains both enzymes, sometimes only one enzyme, sometimes neither enzyme.

Hypoxanthine is oxidised by the tissues to xanthine, and xanthine is oxidised to uric acid (xanthine oxidase). In some animals, but not in man, uric acid is oxidised to allantoin (uricase). The changes may be briefly represented as follows ;—

Nucleic acid

adenine   guanine

hypoxanthine ⟶ xanthine ⟶ uric acid

allantoin
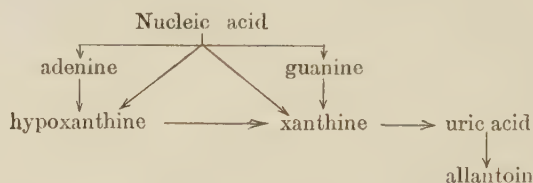
The mechanism of these transformations has been difficult to eluci-date and much confusion as to the origin of uric acid has existed. A full account of the work is given in W. Jones' monograph on "Nucleic Acids."

CHAPTER XXXIV.

NUCLEIC ACIDS.

THE first chemical examination of cell nuclei was made in 1868 by F. Miescher. Pus cells were digested with artificial gastric juice; the protoplasm dissolved and a residue consisting of the more resistant nuclei was left as an insoluble grey powder. This powder was dissolved in dilute sodium carbonate and was precipitated by dilute acetic acid. It was found to contain phosphoric acid and to give the colour tests for proteins. It was named nuclein.

Eight years later Miescher examined the spermatozoa of Rhine salmon. He found that they consisted almost entirely of a salt composed of the base protamine (a protein) and an organic acid which he termed nucleic acid; this acid contained phosphorus.

Nucleins were prepared from other tissues, yeast, red blood corpuscles, etc., by other workers. They were analysed most carefully by Kossel and his pupils to whom our knowledge of the constitution of nucleic acid is almost entirely due. Kossel found that nucleins and also nucleic acid, for which a method of preparation from thymus and other organs was devised by Kossel and Neumann, on hydrolysis by acids gave rise to the purine bases, guanine, xanthine, hypoxanthine and adenine. The two bases, guanine and adenine, have since been shown to be the only ones present in nucleic acid. In addition to the two purine bases three other bases, the three pyrimidine bases, thymine, uracil and cytosine, have been shown to be present in nucleic acid, and besides these compounds there is also present a carbohydrate, a hexose, or a pentose. These compounds are obtained from animal, or plant, nucleic acids. Nucleic acids consist of a carbohydrate, phosphoric acid, two purine bases and two pyrimidine bases, as expressed in the following scheme :—

Animal Nucleic Acid.	*Plant Nucleic Acid.*
Phosphoric acid.	Phosphoric acid.
Hexose (lævulinic acid).	Pentose = *d*-ribose.
Guanine.	Guanine.
Adenine.	Adenine.
Cytosine.	Cytosine.
Thymine.	Uracil.

Plant nucleic acid differs from animal nucleic acid in the nature of the carbohydrate constituent and in the nature of one of the pyrimidine constituents.

It appears that all animal nucleic acids are the same and that all plant nucleic acids are the same.

The constitution of the nucleic acids has not yet been definitely ascertained, but the following formulæ have been provisionally assigned :—

<table>
<tr><td>Animal Nucleic Acid.</td><td>Plant Nucleic Acid [1]</td></tr>
</table>

$$\begin{array}{ll} \text{HO} \\ \quad\diagdown \\ \text{O} = \text{P} - \text{O} - \text{C}_6\text{H}_{10}\text{O}_4 - \text{guanine group.} \\ \quad\diagup \\ \text{HO} \end{array} \qquad \begin{array}{ll} \text{HO} \\ \quad\diagdown \\ \text{O} = \text{P} - \text{O} - \text{C}_5\text{H}_7\text{O}_2 - \text{guanine group.} \\ \quad\diagup \\ \text{HO} \end{array}$$

$$\begin{array}{ll} \text{HO} \\ \quad\diagdown \\ \text{O} = \text{P} - \text{O} - \text{C}_6\text{H}_8\text{O}_2 - \text{thymine group.} \\ \quad\diagup \\ \text{HO} \end{array} \qquad \begin{array}{ll} \text{HO} \\ \quad\diagdown \\ \text{O} = \text{P} - \text{O} - \text{C}_5\text{H}_6\text{O} - \text{cytosine group} \\ \quad\diagup \\ \text{HO} \end{array}$$

$$\begin{array}{ll} \text{HO} \\ \quad\diagdown \\ \text{O} = \text{P} - \text{O} - \text{C}_6\text{H}_8\text{O}_2 - \text{cytosine group.} \\ \quad\diagup \\ \text{HO} \end{array} \qquad \begin{array}{ll} \text{HO} \\ \quad\diagdown \\ \text{O} = \text{P} - \text{O} - \text{C}_5\text{H}_6\text{O} - \text{uracil group.} \\ \quad\diagup \\ \text{HO} \end{array}$$

$$\begin{array}{ll} \text{HO} \\ \quad\diagdown \\ \text{O} = \text{P} - \text{O} - \text{C}_6\text{H}_{10}\text{O}_4 - \text{adenine group.} \\ \quad\diagup \\ \text{HO} \end{array} \qquad \begin{array}{ll} \text{HO} \\ \quad\diagdown \\ \text{O} = \text{P} - \text{O} - \text{C}_5\text{H}_7\text{O}_2 - \text{adenine group.} \\ \quad\diagup \\ \text{HO} \end{array}$$
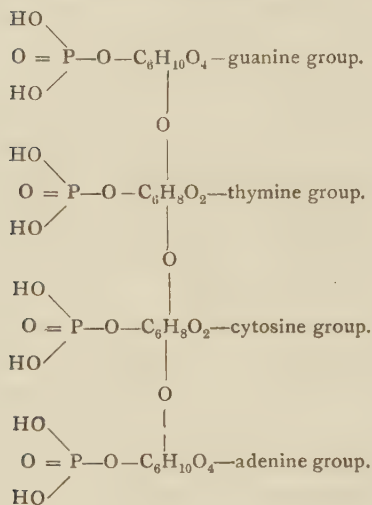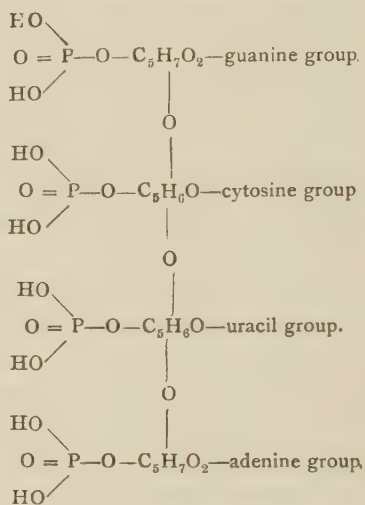
The combination of phosphoric acid with carbohydrate in each case has been proved by the isolation of a carbohydrate ester of phosphoric acid, and the combination of purine base with carbohydrate by the isolation of such a compound. This compound is a glucoside and glucosides of carbohydrate and purine have been synthesised by Emil Fischer. There is as yet no evidence as to how the groups are combined together.

Each of the groupings—phosphoric acid + carbohydrate + purine, or pyrimidine, base—is termed a mononucleotide. Nucleic acid is thus a tetranucleotide. The carbohydrate and purine, or pyrimidine, combination is known as a nucleoside.

a-Nucleoproteins.

If aqueous extracts of various organs be made and these extracts be acidified with acetic acid, a precipitate consisting of protein and nucleic acid is formed. It has been termed nucleoprotein.

[1] Jones and Read, J. Biol. Chem., 1917, **31**, 40.

These *a*-nucleoproteins must be regarded as salts, or as combinations, of nucleic acid and protein; as salts on account of the occurrence of protamine nucleate in spermatozoa, their easy separation, and on account of the property of nucleic acid of precipitating protein from solution when a solution of sodium nucleate in a solution of protein is acidified.

β-Nucleoproteins.   Mononucleotides.

If organs, especially the pancreas, be suspended in water, and raised to the boiling-point, and then filtered from the coagulum of protein, a clear yellow liquid is obtained.   If this liquid be acidified with acetic acid, a precipitate is formed.   This precipitate on purification does not contain protein.   It is a mononucleotide, termed guanylic acid, and consists of guanine, pentose and phosphoric acid.   Another mononucleotide, inosinic acid, has been prepared from meat extract.   It consists of xanthine, pentose and phosphoric acid, and is identical with vernine, a mononucleotide prepared from plants.

These β-nucleoproteins of animals have thus the constitution of plant nucleic acids.   They are not constituents of the nuclei of animal cells, but have been ingested by the tissue from vegetable food.

The work of the various investigators upon nucleic acid is given by Walter Jones in his monograph on " Nucleic Acids."

FURFURANE, THIOPHENE, PYRROLE, AND THEIR DERIVATIVES.

THESE compounds and their derivatives contain heterocyclic rings with 4 atoms of carbon and 1 atom of oxygen, sulphur, or nitrogen. The ring structure in these compounds is represented :—

$$\beta' \; HC\!\!-\!\!CH \; \beta \qquad HC\!\!-\!\!CH \qquad HC\!\!-\!\!CH$$
$$\alpha' \; HC \quad CH \; \alpha \qquad HC \quad CH \qquad HC \quad CH$$
$$O \qquad\qquad S \qquad\qquad NH$$

Furfurane.   Thiophene.   Pyrrole.
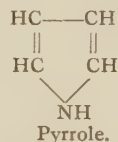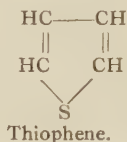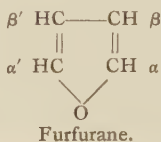
The hydrogen atoms in these compounds may be substituted by other atoms, or groups. Two mono-substituted products α and β (α' and β') are possible. If more hydrogen atoms be substituted, the derivatives are $\alpha\alpha'$, or $\alpha\beta'$, or as $\alpha\gamma$ or $\alpha\delta$, if the lettering, or numbering, be continued consecutively round the ring. The fixed point from which the lettering, or numbering, starts is the O, S, or N atom.

FURFURANE AND ITS DERIVATIVES.

The origin, main interest, and importance of the furfurane compounds is in the fact that they are formed from carbohydrates. The following compounds are the chief derivatives :—

$$HC\!\!-\!\!CH \qquad HC\!\!-\!\!CH \qquad HC\!\!-\!\!CH$$
$$HC \quad C.CH_2OH \qquad HC \quad C.CHO \qquad HC \quad C.COOH$$
$$O \qquad\qquad O \qquad\qquad O$$

Furfur-   Furfur-   Pyromucic
alcohol.   aldehyde,   acid.
     furfural
     (furfurol).

$$HC\!\!-\!\!CH \qquad\qquad HC\!\!-\!\!CH$$
$$CH_3.C \quad C.CHO \qquad CH_2OH\!\!-\!\!C \quad C.CHO$$
$$O \qquad\qquad\qquad O$$

α', or ω-methyl- α', or ω-hydroxymethyl
furfural.   furfural.
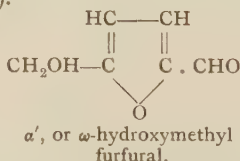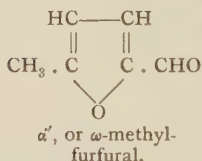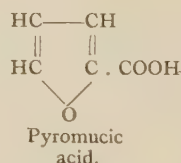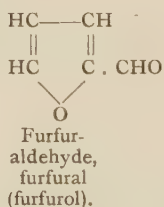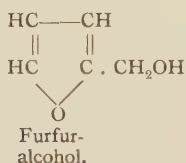
Furfurane, C_4H_4O, is obtained by the distillation of the barium salt of pyromucic acid and is contained in the tar from pinewood. Furfurane is a liquid with a peculiar smell. It boils at $32°$ and is insoluble in

water. It is reduced to tetrahydrofurfurane when passed over zinc dust, or nickel dust, heated to 170°. It reacts violently with concentrated hydrochloric acid forming a brown amorphous substance, and gives a purplish colour reaction with sulphuric acid and isatin, or phenanthraquinone.

Furfuralcohol, $C_4H_3O . CH_2OH$, is formed from furfuraldehyde by reduction, or by the action of caustic soda. It is present in the oil from roasted coffee. It is a colourless liquid, which boils at 171°, and is easily soluble in water. In solution it rapidly resinifies. It gives a blue-green colour to a pinewood shaving moistened with hydrochloric acid.

Furfuraldehyde, $C_4H_3O . CHO$.

Furfuraldehyde, or furfural, is the chief compound of the group and is formed from bran and other carbohydrates by distillation with dilute sulphuric acid. It is formed quantitatively by the distillation of pentoses with dilute acid and therefore serves in their estimation :—

$$\begin{array}{l} CH_2OH \quad CHOH . CHO \\ | \qquad\quad | \\ CHOH - CHOH \end{array} = 3H_2O + \begin{array}{l} \qquad O \\ \quad\diagup\diagdown \\ HC \quad C . CHO \\ \| \qquad \| \\ HC - CH \end{array}$$
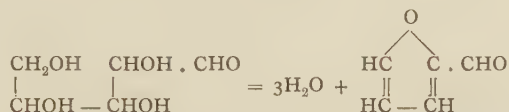
It is a colourless liquid, which boils at 162°, and has a peculiar aromatic smell. It turns brown in the air, is easily soluble in alcohol, but only slightly soluble in water.

It has all the properties of an aromatic aldehyde forming an oxime, a hydrazone, etc. By alkali it is converted into a mixture of alcohol and aldehyde. It condenses with numerous other compounds. It gives colour reactions with a-naphthol and other phenols which serve in testing for carbohydrates.

Pyromucic Acid, $C_4H_3O . COOH$, is obtained by the dry distillation of mucic acid, or by the oxidation of furfural. It is a liquid which boils at 134°.

a'-**Methyl-furfuraldehyde,** $C_4H_2O . CH_3 . CHO$, is formed by the distillation of methyl pentoses with hydrochloric acid and resembles furfural.

ω-**Hydroxymethyl - furfuraldehyde,** $C_4H_2O . CH_2OH . CHO$, is formed in small quantities from hexoses, especially ketoses, by the action of concentrated acids. It resembles furfural. Molisch's reaction for carbohydrates (p. 260) is due to this substance.

THIOPHENE.

Thiophene, C_4H_4S, was discovered in the benzene from coal tar from which it was obtained by extraction with concentrated sulphuric acid.

Thiophene resembles benzene in its reactions more closely than furfurane and pyrrole, but as yet neither thiophene, nor its derivatives, have been obtained from natural substances.

PYRROLE AND ITS DERIVATIVES.

Pyrrole and its derivatives are closely connected with the proteins and with the two respiratory pigments, chlorophyll and hæmoglobin. A pyrrole nucleus is present in the molecule of nicotine, of cocaine, and of other alkaloids (p. 356). We have to consider the following compounds in connection with the constitution of the proteins and the complex chlorophyll and hæmoglobin molecules :—

$$
\begin{array}{ccc}
\text{HC} \!\!-\!\! \text{CH} & \text{H}_2\text{C} \!\!-\!\! \text{CH} & \text{H}_2\text{C} \!\!-\!\! \text{CH}_2 \\
\| \quad \| & | \quad \| & | \quad | \\
\text{HC} \quad \text{CH} & \text{H}_2\text{C} \quad \text{CH} & \text{H}_2\text{C} \quad \text{CH}_2 \\
\diagdown\!\diagup & \diagdown\!\diagup & \diagdown\!\diagup \\
\text{NH} & \text{NH} & \text{NH} \\
\text{Pyrrole.} & \text{Pyrroline.} & \text{Pyrrolidine.}
\end{array}
$$

$$
\begin{array}{cc}
\text{H}_2\text{C} \!\!-\!\! \text{CH}_2 & \text{HO . CH} \!\!-\!\! \text{CH}_2 \\
| \quad | & | \quad | \\
\text{H}_2\text{C} \quad \text{CH . COOH} & \text{H}_2\text{C} \quad \text{CH . COOH} \\
\diagdown\!\diagup & \diagdown\!\diagup \\
\text{NH} & \text{NH}
\end{array}
$$

<div style="display:flex">
α-pyrrolidine-
carboxylic acid,
or
proline.

Hydroxyproline,
or
γ-hydroxy-α-pyrrolidine
carboxylic acid.
</div>

$$
\begin{array}{cc}
\text{CH}_3 . \text{C} \!\!-\!\! \text{C} . \text{C}_2\text{H}_5 & \text{CH}_3 . \text{C} \!\!-\!\! \text{C} . \text{C}_2\text{H}_5 \\
\| \quad \| & \| \quad \| \\
\text{CH}_3 . \text{C} \quad \text{C . H} & \text{H . C} \quad \text{C . CH}_3 \\
\diagdown\!\diagup & \diagdown\!\diagup \\
\text{NH} & \text{NH}
\end{array}
$$

<div style="display:flex">
Isohæmopyrrole
α, β-dimethyl,
β'-ethyl-pyrrole.

Kryptopyrrole
α-methyl,
β'-methyl-β-ethyl
pyrrole.
</div>

$$
\begin{array}{cc}
\text{CH}_3 . \text{C} \!\!-\!\! \text{C} . \text{C}_2\text{H}_5 & \text{C}_2\text{H}_5 . \text{C} \!\!-\!\! \text{C} \!\!-\!\! \text{CH}_2 . \text{CH}_2 . \text{COOH} \\
\| \quad \| & \| \quad \| \\
\text{CH}_3 . \text{C} \quad \text{C . CH}_3 & \text{CH}_3 . \text{C} \quad \text{CH} \\
\diagdown\!\diagup & \diagdown\!\diagup \\
\text{NH} & \text{NH}
\end{array}
$$

<div style="display:flex">
Phyllopyrrole
or
α, α'-dimethyl,
β-methyl-β'-ethyl
pyrro'e.

Isophonopyrrole-
carboxylic acid,
or
α-methyl-β-ethyl-
pyrrole-
β'-propionic acid.
</div>

Pyrrole. C_4H_5N.

Pyrrole was found in coal tar in 1834 and in bone oil in 1858 and is usually obtained from bone oil.

Preparation.

The oil which is obtained by the dry distillation of bones contains pyridine and basic substances, aromatic hydrocarbons, pyrrole and its homo-

logues, but consists mainly of the nitriles of fatty acids.   The basic substances are removed by agitation with dilute acid, the nitriles are hydrolysed by boiling with alkali, and the oil which remains is fractionally distilled.   The fraction passing over between 115 and 130° contains the pyrrole.   By boiling with solid caustic potash it is converted into solid potassium pyrrole, C_4H_4NK, which is filtered off and decomposed by water.   The pyrrole is then isolated by distillation.

Fat-free bone gelatin is said to give a distillate consisting mainly of pyrrole and its homologues.

It is probably formed from the proline and hydroxyproline contained in the protein, but may also arise by the dry distillation of glutamic acid.

It has been synthesised by passing acetylene and ammonia through red-hot tubes, by the dry distillation of the ammonium salt of mucic acid, and by the reduction of succinimide by distillation over zinc dust :—

$$CH_2-CO\diagdown NH + 2H_2 = CH=CH\diagdown NH + 2H_2O.$$
$$CH_2-CO\diagup \qquad\qquad CH=CH\diagup$$
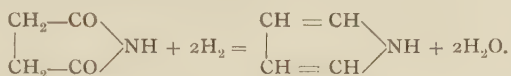
Properties.

Pyrrole is a colourless liquid smelling like chloroform, but turning brown in the air.   It boils at 131°, is very slightly soluble in water, but is easily soluble in alcohol and ether.

Pyrrole is a secondary amine and has a slight basic character ; it dissolves slowly in dilute acids and is converted into a resin by strong acids.   Its solution in dilute acids, on warming, deposits a red precipitate termed pyrrole red.

It gives a fiery red colour with a pine shaving moistened with hydrochloric acid.   Hence its name from πυρρος.

As a secondary amine, pyrrole forms a nitroso compound with sodium ethoxide and amyl nitrite.

Potassium dissolves in pyrrole with evolution of hydrogen.   The combination of pyrrole with potassium to form solid potassium pyrrole is probably due to the acid influence of the CH groups.

The pyrrole ring is easily ruptured.   Succinyl dialdoxime is formed by the action of hydroxylamine upon pyrrole. a-substituted pyrroles yield ketoximes, β-substituted pyrroles yield aldoximes from which dibasic acids can be obtained.   This reaction serves for determining the position of substituting groups.

Pyrrole reacts violently with halogens, but derivatives are obtained by using dilute solutions.   Tetra-iodopyrrole, which is prepared by the action of iodine on pyrrole in the presence of alkali, forms yellow-brown prisms which melt at 140°.   Under the name of iodol it is used as an antiseptic and has an advantage over iodoform in possessing no smell.

Pyrroline and Pyrrolidine.

Pyrrole is easily reduced by zinc and acetic acid, or by electrolysis, to pyrroline ; it is converted into pyrrolidine by hydriodic acid, or by passing pyrrole and hydrogen over nickel dust heated to 190°.

Pyrroline is a liquid boiling at 91° and has an ammoniacal smell. It is a strong base and forms stable salts with acids.

Pyrrolidine is formed by loss of ammonia on heating putrescine hydrochloride :—

$$\begin{matrix} CH_2\text{---}CH_2 . NH_2 \\ | \\ CH_2 . CH_2 . NH_2 \end{matrix} = NH_3 + \begin{matrix} CH_2 . CH_2 \\ | \qquad\qquad \\ CH_2 . CH_2 \end{matrix}\!\!\!\!> NH.$$
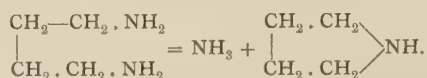
Pyrrolidine is a liquid which has a smell resembling pepper. It boils at 87° and, like pyrroline, is a strong base.

Proline and Hydroxyproline.

These compounds are constituents of proteins. They result from the hydrolysis of proteins by acids, or alkalies. A complex process of separation is required to isolate them from proteins (see "Chemical Constitution of the Proteins"). They differ from other units of the protein molecule by being easily soluble in alcohol (cf. glycine, p. 168).

Alkyl Derivatives of Pyrrole.

Derivatives of pyrrole are easily prepared from potassium pyrrole. Potassium pyrrole reacts with alkyl halides, acid chlorides, etc., to form derivatives in which the substituting group is attached to the nitrogen atom :—

$$\begin{matrix} CH = CH \\ | \qquad\qquad \\ CH = CH \end{matrix}\!\!\!\!> NK + CH_3I = \begin{matrix} CH = CH \\ | \qquad\qquad \\ CH = CH \end{matrix}\!\!\!\!> N . CH_3 + KI.$$
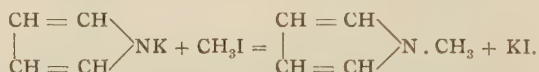
On heating, these compounds undergo rearrangement; the substituting group changes its position and attaches itself to a carbon atom.

Isohæmopyrrole, kryptopyrrole, phyllopyrrole and other alkyl pyrroles are formed by the reduction of hæmin, chlorophyll and bile pigments. A mixture is obtained from which the individual compounds are separated.

Hæmoglobin and Chlorophyll.

Both hæmatin, the constituent imparting the colour to hæmoglobin, the red pigment of the blood, and chlorophyll, the green pigment of plants, are complex compounds containing pyrrole nuclei.

On reduction, both give phyllopyrrole, isohæmopyrrole and kryptopyrrole, which indicate the presence of three pyrrole nuclei.

On oxidation, both yield methyl-ethyl-maleinimide and hæmatinic acid :—

$$\begin{matrix} CH_3 . C\text{---}CO \\ \| \qquad\qquad \\ C_2H_5 . C\text{---}CO \end{matrix}\!\!\!\!> NH \qquad\qquad \begin{matrix} CH_3 . C\text{---}CO \\ \| \qquad\qquad \\ HOOC . CH_2 . CH_2 . C\text{---}CO \end{matrix}\!\!\!\!> NH.$$
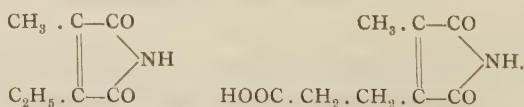
The yield of these compounds indicates the presence of four pyrrole nuclei.

The bile pigment, bilirubin, is also closely related to hæmatin.   All these compounds can ultimately be converted into aetioporphyrin, $C_{31}H_{36}N_4$, which probably has the formula :—
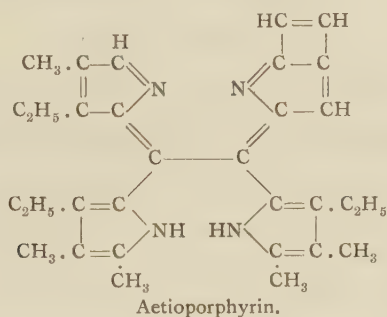
Aetioporphyrin.

Four pyrrole nuclei are thus present in aetioporphorin.   Hæmatin and chlorophyll are more complex ; they contain the aetioporphyrin structure.

Chlorophyll really consists of two compounds, chlorophyll *a* and chlorophyll *b*.   Both contain magnesium in organic combination, and both are esters of an acid termed chlorophyllin with methyl alcohol and phytol.   Alkali changes chlorophyllin ultimately to aetiophyllin. Chlorophyllin and all its derivatives lose their magnesium by the action of dilute acids.   Aetiophyllin gives aetioporphyrin, and is its magnesium compound.

Hæmatin forms a hydrochloride, termed hæmin, and contains iron in organic combination.   Hæmatoporphyrin is formed from hæmatin and does not contain iron.   Hæmatoporphyrin yields firstly hæmophorphyrin and then aetioporphorin.

PYRIDINE AND ITS DERIVATIVES.

THE six-membered heterocyclic ring compounds containing 5 atoms of carbon and 1 atom of nitrogen, of which pyridine is the simplest member and from which all the other compounds of the group can be derived, resemble the benzene compounds very closely. The simpler members are present in coal tar and bone oil and are formed by the oxidation of the complex alkaloids which occur in plants.

The Structure of Pyridine.

The empirical formula of pyridine, C_5H_5N, points to its not being an open chain compound. For reasons similar to those which led to the adoption of a closed ring structure for the constitution of benzene and from the great similarity which pyridine has to benzene in its reactions, the following ring structure has been assigned to pyridine:—
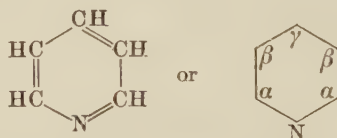
This structure shows that pyridine is a tertiary base, that three isomeric monosubstitution and six disubstitution derivatives can be derived from it; in general it expresses all the facts known about pyridine and its derivatives.

Pyridine.

Pyridine was first obtained from bone oil, but is contained in coal tar from which it is usually prepared.

Preparation.

The acid solution, or liquor, which results in the purification of benzene and its homologues from coal tar is treated with sodium hydroxide; the basic substances separate out as oils. This oil consists of a mixture of pyridine, its homologues, quinoline and other substances. These constituents can be partially separated by fractional distillation, but the pure compounds are finally isolated by the fractional crystallisation of their salts. The bases are liberated from the salt by alkali and purified by distillation.

Bone oil is extracted with sulphuric acid; the bases are separated by sodium hydroxide, distilled and purified as described above.

Properties.

Pyridine is a colourless liquid which boils at 115° and has a specific gravity of 1·003 at 0°. It has a pungent characteristic and disagreeable odour and mixes with water in all proportions.

It is a strong base, which can turn red litmus blue, and forms salts with acids.

Pyridine is a tertiary amine as shown by its negative behaviour to nitrous acid and the fact that it combines with alkyl halides to form pyridine alkyl halides, such as pyridine methiodide, $C_5H_5N . CH_3I$.

On heating, the methyl group attached to the N atom changes its position and a-alkyl pyridines are formed.

This reaction may be used as a test for pyridine :—

If a few drops of pyridine be heated with a few drops of methyl iodide, a violent reaction takes place and pyridine methiodide is formed. On adding a small quantity of solid potash and again heating, the compound is decomposed and the disagreeable smell of methyl pyridine hydroxide will be noticed.

Pyridine is not oxidised by nitric acid, or chromic acid, and it is only slowly attacked by the halogens and sulphuric acid, forming substitution products.

Piperidine, $C_5H_{10}NH$.

Piperidine is a constituent of the alkaloid, piperine, from which it is obtained by hydrolysis with alkali.

It is formed by the reduction of pyridine with sodium and alcohol, and it is converted into pyridine by heating with concentrated sulphuric acid at 300° :—
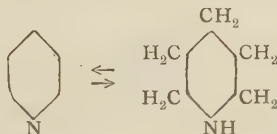
Piperidine is formed by the dry distillation of pentamethylene diamine hydrochloride :—
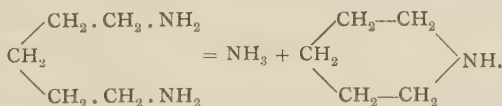
Preparation.

Powdered pepper is extracted with alcohol; the extract is evaporated to dryness and the residue is distilled with soda. The alkaline distillate is neutralised with hydrochloric acid and evaporated to dryness. The residue which consists of ammonium chloride and piperidine hydrochloride is treated

with hot alcohol. The solution containing the piperidine hydrochloride is evaporated and distilled with soda and the oil which passes over is purified by distillation.

Properties.

Piperidine is a colourless liquid which boils at 106°. It has the pungent smell of pepper and mixes with water. It is a strong base and a secondary amine, forming nitrosopiperidine with nitrous acid.

Homologues of Pyridine.

The following homologues of pyridine, mixed with pyridine, are contained in the basic fraction of coal tar and bone oil and are separated by fractional distillation as stated under pyridine.

I. The monomethyl pyridines, or picolines :—

α-methylpyridine,	β-methylpyridine,	γ-methylpyridine,
or	or	or
α-picoline.	β-picoline.	γ-picoline.
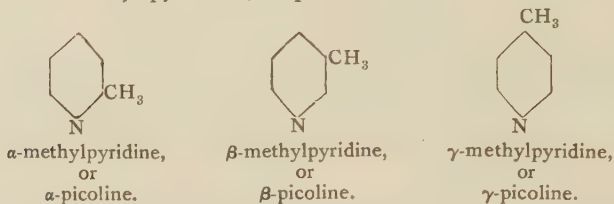
II. The dimethyl pyridines, or lutidines.
III. The trimethyl pyridines, or collidines.
They closely resemble pyridine in properties.

Pyridine Carboxylic Acids.

The side chain of the methyl pyridines, on oxidation, is converted into carboxyl and the pyridine carboxylic acids are obtained :—

| Picolinic acid. | Nicotinic acid. | Isonicotinic acid. |
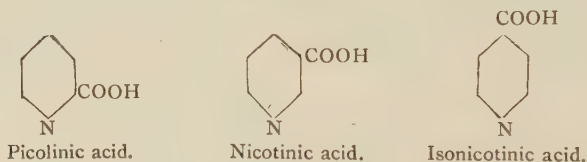
These compounds are white crystalline solids soluble in water. They possess basic properties and acidic properties, forming salts with acids and bases.

Nicotinic Acid is formed by the oxidation of nicotine (p. 358). Nicotine therefore contains a substituting group in the β-position of the ring.

Picolinic Acid gives a red coloration with ferrous sulphate. This reaction is given by all acids derived from pyridine containing a carboxyl group in the α-position.

Quinolinic Acid is formed by the oxidation of quinoline with permanganate. Quinolinic acid is a dibasic acid having the constitution :—
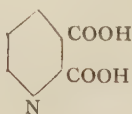
It is a crystalline solid, soluble with difficulty in water. It gives an orange coloration with ferrous sulphate which shows that one carboxyl group is in the α-position. It is converted into nicotinic acid when it is heated to 190°. These reactions show the position of the carboxyl groups.

QUINOLINE AND ISOQUINOLINE.

These compounds have the empirical formula C_9H_7N and are present in coal tar and bone oil. Their constitution is expressed by the formulæ :—

Quinoline.

Isoquinoline.

The presence of the pyridine ring in these compounds is shown by their oxidation. Quinoline gives quinolinic acid : isoquinoline gives β,γ-pyridine dicarboxylic acid, or cinchomeronic acid, and phthalic acid. The formulæ of both compounds have been proved by synthesis.

Quinoline is usually prepared by synthesis. It is a colourless oily liquid which boils at 239° and has a specific gravity of 1·095 at 20°. It has a peculiar and pleasant smell and is only slightly soluble in water.

Isoquinoline is prepared from the fraction of coal tar, or bone oil, which distils between 236° and 243°. The bases are converted into sulphates and fractionally crystallised from alcohol. The sulphate is decomposed by potash and the base distilled.

Isoquinoline is a colourless solid which melts at 23°, boils at 241° and closely resembles quinoline.

Both compounds are tertiary amines and form salts with acids. They are stable ring compounds resembling naphthalene and pyridine.

Quinine and isoquinoline nuclei are contained in the molecules of the more complex alkaloids.

CHAPTER XXXVII.

HYDRO-AROMATIC COMPOUNDS.

BENZENE, its homologues and derivatives, though they form a special group of compounds with special properties, behave nevertheless in some respects like unsaturated compounds. They can be reduced under certain conditions and they will combine by addition with the halogens on exposure to sunlight. The reduced compounds are known as the hydro-aromatic compounds, and the halogen addition compounds are regarded as derived by substitution from reduced benzene. The aromatic compounds are most easily reduced by the method of Sabatier, which consists in passing their vapour mixed with hydrogen over nickel dust heated to about 170°.

Benzene gives three reduction products :—
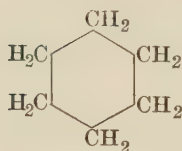
Hexahydrobenzene,
or
Hexamethylene, or
Cyclohexane.
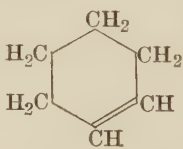
Tetrahydrobenzene,
or
Cyclohexene.
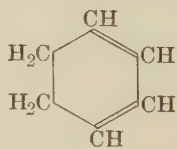
Dihydrobenzene,
or
Cyclohexadiene.

These compounds have properties like the aliphatic compounds. Hexahydrobenzene, on oxidation, is reconverted into benzene.

The natural compounds, the inositols, the terpenes, the sterols and cholalic acid are hydro-aromatic compounds.

THE INOSITOLS.

The inositols are hydroxy-derivatives of hexahydrobenzene.

Quercitol, or cyclohexanepentol, is found in acorns and in the leaves of *Chamærops humilis*, a variety of palm. It is a colourless solid which melts at 235° and is dextro-rotatory, $[a]_D = + 24.16°$. A lævorotatory quercitol has been found in the leaves of *Gymnema sylvestre*. It melts at 174° and has $[a]_D = - 73.9°$.
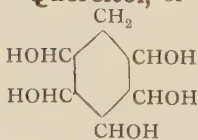
Inositol. Several varieties of inositol have been found in nature:—an inactive form, 2 active forms and a racemic form. Seven inactive forms are theoretically capable of existence.

CHOH

HOHC⟨⟩CHOH

HOHC⟨⟩CHOH

CHOH
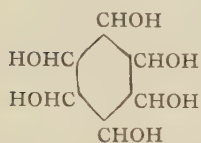
i-Inositol is found in heart-muscle and other animal organs, but is present in larger amounts in unripe beans and peas. It is present in the free state and also in combination with phosphoric acid as ester in the husks of various cereals. The calcium and magnesium salt of this acid is termed *Phytin*.

d-Inositol is obtained by the reduction of pinitol with hydriodic acid.

l-Inositol is obtained from quebrachitol by reduction.

Scyllitol, an inactive inositol, is present in the organs of various elasmobranch fish—the dog fish, skate and shark.

Cocositol has been isolated from the leaves of cocos and closely resembles *i*-inositol.

Pinitol is monomethyl *d*-inositol.

Quebrachitol is monomethyl *l*-inositol.

THE TERPENES.

Nearly all parts of plants contain volatile substances with a highly characteristic and pleasant smell. These substances are the essential oils, e.g. oil of turpentine, oil of lemons, etc. The various kinds of camphor, which are crystalline solids, the resins and india-rubber are closely related substances.

They are prepared from plants by steam distillation, by pressing, or by extraction with organic solvents. Besides their use in perfumery, in making essences, they are used in the preparation of oil paints, varnishes, etc. Several are used in medicine.

The essential oils are generally complex mixtures, the main constituent imparting the characteristic properties; several essential oils may contain the same constituent and yet differ in smell on account of the presence of different highly odoriferous substances. Oil of turpentine exists in the greatest quantity. It flows from the stems of pine trees when incisions are made in the surface and consists of solids dissolved in the liquid. Crude oil of turpentine is separated by steam distillation, the solids remaining behind and constituting colophony, or resin.

The chief constituent of oil of turpentine is pinene. Limonene is present in oil of lemon. They are colourless, very refractive liquids boiling between 150° and 180°. Camphene is solid. They are insoluble in water, but soluble in most organic liquids. They are good solvents, dissolving resin, caoutchouc, iodine, phosphorus and sulphur. The majority are optically active; sometimes both the dextro and lævo forms are found in nature, and the inactive mixture of some of them has been prepared.

Reactions.

(1) They easily polymerise to form resinous substances.
(2) On exposure to air, or oxygen, they are oxidised and yield resins.
(3) On oxidation with permanganate, etc., they are converted into benzene derivatives.
(4) On treatment with ozone, they form ozonides.
(5) On reduction, they are converted into hydroterpenes.
(6) They combine with bromine and halogen acids to form addition compounds, which are frequently crystalline solids.
(7) They react with nitrosyl chloride, NOCl.

Constitution of the Members of the Terpene Group.

Most of the members of the terpene group are unsaturated hydrocarbons of the formula $C_{10}H_{16}$; others, such as camphor, are alcoholic, or ketonic, derivatives and possess the empirical formulæ $C_{10}H_{16}O$, $C_{10}H_{18}O$, $C_{10}H_{20}O$.

Though most of the hydrocarbons have the empirical formula $C_{10}H_{16}$, several have the formulæ $C_{15}H_{24}$, $C_{20}H_{32}$ and $(C_5H_8)_n$. The unsaturated hydrocarbon isoprene, C_5H_8, has the same percentage composition and is obtained by the distillation of caoutchouc; it polymerises to a hydrocarbon $C_{10}H_{16}$ and can be made to polymerise to caoutchouc, the constituent of rubber. The group may therefore be divided into :—

(1) Hemiterpenes C_5H_8 (4) Diterpenes $C_{20}H_{32}$
(2) Terpenes $C_{10}H_{16}$ (5) Polyterpenes $(C_5H_8)_n$
(3) Sesquiterpenes $C_{15}H_{24}$ and their derivatives.

The structure of most of the compounds has been established and many of the natural ones have been prepared by synthesis.

A few are open chain compounds, e.g.—

Geraniol $\quad \underset{CH_3}{\overset{CH_3}{>}} C=CH . CH_2 . CH_2 . C \underset{CH . CH_2OH}{\overset{CH_3}{<}}$

Citral geraniol $\quad \underset{CH_3}{\overset{CH_3}{>}} C=CH . CH_2 . CH_2 . C \underset{CH . CHO.}{\overset{CH_3}{<}}$
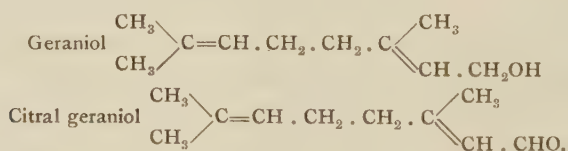
Geraniol makes up about 90 per cent. of Indian geranium oil. Citral is present in oil of lemons, orange, etc., and is obtained by oxidising geraniol.

Most of the terpenes are hydro-aromatic compounds derived from cymene. They are most conveniently regarded as unsaturated hydrocarbons derived from hexahydrocymene, or menthane,

and possessing the skeleton
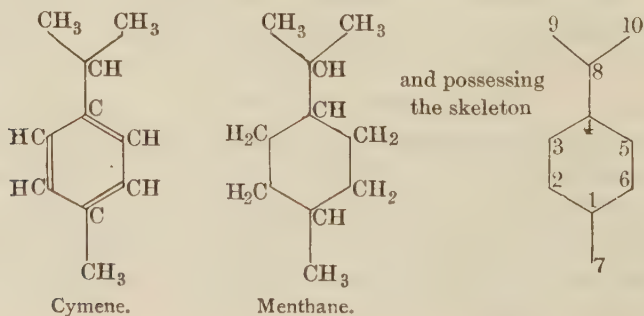
Cymene.   Menthane.

Menthane Group.

Menthol and a few other terpenes are derivatives of menthane, e.g. :—

Menthol. Menthone. Carvomenthol. Carvomenthone.
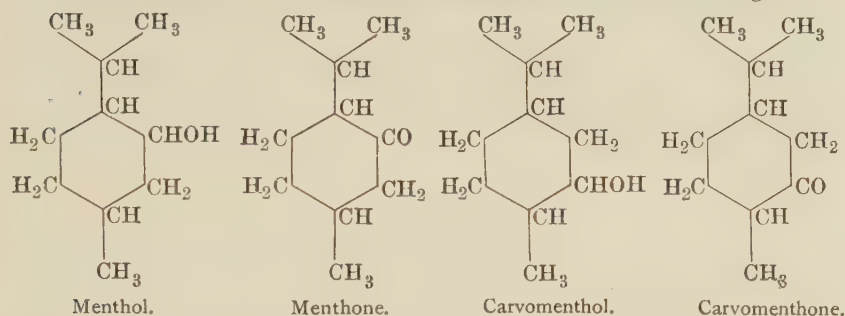
Menthol is the chief constituent of peppermint oil and can be made by reducing menthone.

Menthone is present in Japanese, American and Russian peppermint oil. It exists in two optically active forms.

Menthene Group, $C_{10}H_{18}$.

Several terpenes are derivatives of menthene, or carvomenthene, and contain one double bond in the molecule, e.g. :—

Menthene. Carvomenthene. α-Terpineol. Terpinenol.
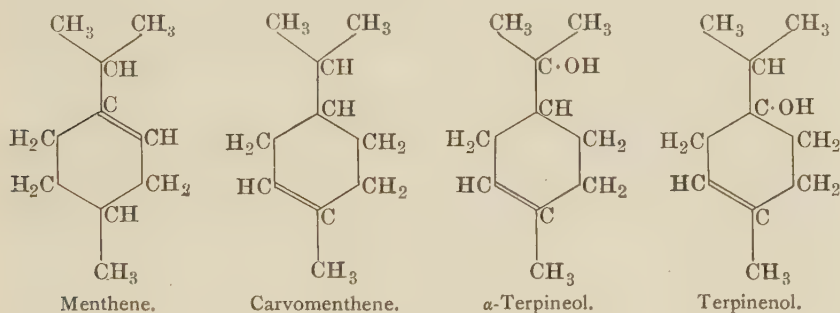
Menthadiene Group.

Two double bonds are present in the molecules of these terpenes, e.g. :—

d and l-limonene, α-Phellandrene. β-Phellandrene. Carvone.
d, l-limonene,
or
dipentene.
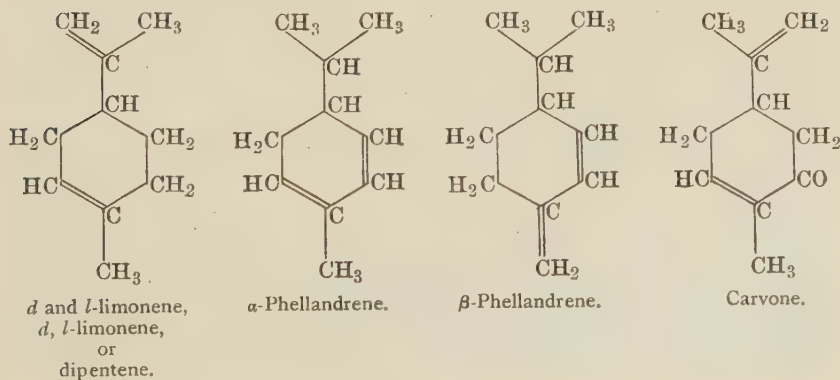
d-Limonene is found in numerous essential oils, in that of lemon, bergamot, kummel, dill, celery.

l-Limonene is present in pine-needle oil and Russian peppermint oil.

Dipentene, or d, l-limonene, is present in Russian and Swedish turpentine which has been heated to a high temperature. It is also formed by heating other terpenes. It is contained in the distillate from rubber, having been formed by the polymerisation of isoprene.

Phellandrene is present in fennel oil. Carvone is present in oil of kummel and oil of dill.

Pinane and Camphane Groups.

These groups have the isopropyl group attached also to another carbon atom of the ring, e.g. :—
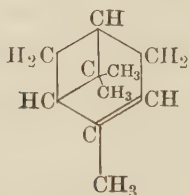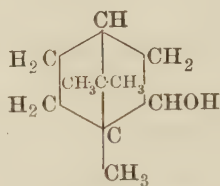
d and l-Pinene.	Borneol, or Borneo camphor.	Camphor, or Japan camphor.
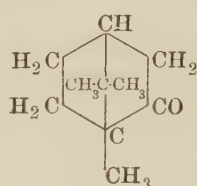
d-Pinene is the chief constituent of American, Algerian and Greek turpentine, l-pinene of French and Spanish turpentine; both are prepared by fractional distillation of turpentine.

Borneol occurs as d-borneol in *Dryobalanops camphora*, which is grown in Borneo and Sumatra, as l-borneol in the oil of *Blumea balsamifera*. As ester with fatty acids it occurs in pine-needle oil. It is very like Japan camphor, but has also a peppermint smell.

d-Camphor, or Japan camphor, occurs in the camphor tree, *Cinnamomum camphora*, and is obtained by distillation and sublimation. It can be made artificially from the pinene in turpentine. It is a colourless, transparent, tough mass, which crystallises from alcohol and is very volatile, and is used in making celluloid and smokeless gunpowder.

It seems most likely that all the terpenes are made by the condensation of isoprene by the action of acids in the plant juices, and it is most remarkable that so many different isomers can be formed from the unsaturated isoprene. The method of formation of isoprene in plants is unknown, but it may arise by removal of carbon dioxide and ammonia from leucine and isoleucine.

Sesquiterpenes are present in the various essential oils. They are yellowish, viscous liquids boiling between 250-280° with slight but not pleasant smell, and they easily change into resins.

The diterpenes and polyterpenes are also yellow viscous liquids boiling above 300° and not easily volatile with steam. They are found in balsams and resins.

The resins occur in the plant oils and are also formed from the terpenes by oxidation in the air. Their solutions in the terpenes are generally called balsams; the solid resins are amorphous shining substances. They consist of a mixture of resin acids and dissolve in alkalies, from which they are precipitated by acids. They yield various aromatic compounds by fusion with potash and on reduction yield benzene, naphthalene, etc.

Caoutchouc, the constituent of rubber, is particularly important industrially. The substance which forms caoutchouc can be extracted by ether from the plant juice, and on exposure to light, or by action of acids, it polymerises to rubber. Pure caoutchouc is soluble in benzene, carbon disulphide, chloroform, etc. · It is acted upon by ozone giving a diozonide.

It can take up sulphur by kneading with sulphur, or by treating with sulphur dissolved in sulphur chloride, and this combination constitutes rubber, ebonite.

The colour of rubber depends on whether lead oxide, antimony oxide, etc., has been used in the vulcanising process.

THE STEROLS.

Cholesterol, $C_{27}H_{45}OH$.

Cholesterol was discovered in bile and has been found in the bile of all animals with one exception. It has since been shown to be present in small quantities in blood and the tissues of man and animals ; in somewhat large quantities it is present in bone marrow and nervous tissue. It is very seldom found in urine and, when found, only in the smallest quantities. Crystalline deposits of cholesterol are found in pathological effusions, in pus and in diseased arteries. Gallstones usually consist almost entirely of cholesterol. Not only is cholesterol present as such, but also in the form of its esters with the higher fatty acids. Lanolin, or wool fat, is composed mainly of esters of cholesterol.

Constitution.

Cholesterol has been shown to be a secondary alcohol and to contain one unsaturated bond. It possesses a very complex structure, containing at least two hydro-aromatic rings. Its formula according to Windaus is the following :—
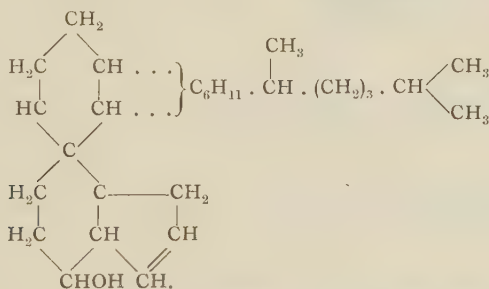
The structure of C_6H_{11} has still to be determined.

Preparation.

* (1) *From Gallstones.*

Cholesterol is most easily prepared from gallstones. The powdered stone is extracted with a mixture of ether and alcohol. The filtered solution, on evaporation, leaves a residue of cholesterol. It is purified

by boiling with alcoholic potash, the solution is evaporated to dryness and the residue extracted with ether. The crystals obtained after evaporation of the ether are recrystallised from alcohol.

(2) *From Brain.*

Sheep's brain is dried, or ground up with some sand and about 3 parts of plaster of Paris. The dry material is powdered and covered with acetone. The acetone is filtered off and the mass again treated with acetone. On distilling off the acetone, crystals of cholesterol remain. They may be purified as above.

Properties.

Cholesterol forms a white crystalline solid melting at 147°. It crystallises in needles from ether, benzene, etc.; in characteristic four-sided plates with a notched angle and containing 1 molecule of water of crystallisation (Fig. 45) from aqueous alcohol. It is insoluble in water and soluble with difficulty in cold alcohol. In hot alcohol, ether, acetone, chloroform and other organic solvents it is readily soluble.
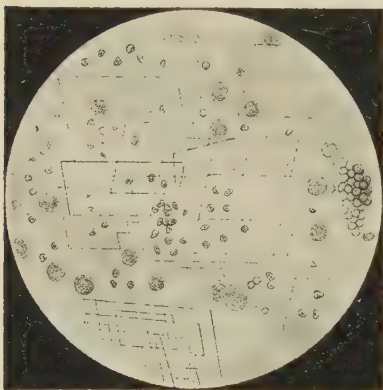
Fig. 45.—Cholesterol.   (After Funke.)

The unsaturated character of cholesterol is shown by the formation of addition compounds with the halogens and halogen acids.

As an alcohol, cholesterol forms esters. The acetate and benzoate are very characteristic and serve to distinguish cholesterol from phytosterols (below).

Cholesteryl Acetate.—A small quantity of dry cholesterol is boiled with 2 or 3 c.c. of acetic anhydride for 1 or 2 minutes. The acetic anhydride is evaporated, or the solution is poured into water. The residue, or precipitate, is crystallised from dilute alcohol. It melts at 114°.

Cholesteryl Benzoate.—Cholesterol is boiled with benzoyl chloride for a few minutes and the solution poured into alcohol. The precipitate of cholesteryl benzoate is recrystallised from hot alcohol. This compound melts at 145° to a turbid liquid, which becomes clear at 178-180°; on cooling, it exhibits a play of colours, of which blue is the most marked.

Esters of cholesterol with palmitic, oleic and other acids have also been prepared.

On careful oxidation, cholesterol yields a ketone, cholestenone.

Tests.

* (1) Crystalline form of crystals separated from alcohol.

* (2) On running a drop of sulphuric acid (5 vols. of conc. acid to 1 vol. of water) upon some crystals on a glass slide, covered with a cover slip, the crystals become red. A drop of iodine solution placed against the cover slip and brought into contact with the crystals by drawing it through with filter paper, changes the colour of the crystals at the points of contact to violet, blue and black.

* (3) *Salkowski's Reaction.*—A minute quantity of dry cholesterol is dissolved in a little chloroform in a dry test tube. An equal volume of concentrated sulphuric acid is added and the liquids mixed. The chloroform rises to the surface coloured at first red, then purple, and the sulphuric acid is yellow and shows a green fluorescence. If the chloroform be poured into a basin, it becomes blue, green and yellow. It is decolorised if water be added, but the colour returns on adding strong sulphuric acid. The colour is only stable in the presence of acid. If the sulphuric acid be diluted with glacial acetic acid, it becomes red, but still shows a green fluorescence.

* (4) *Liebermann's Reaction.*—A crystal of cholesterol is dissolved in about 2 c.c. of chloroform in a dry test tube, 2 or 3 drops of acetic anhydride are added and, drop by drop, concentrated sulphuric acid. A red colour, which becomes blue and finally bluish green, is formed.

Isocholesterol, $C_{27}H_{46}O$.

Isocholesterol has been found together with cholesterol in lanolin.

Coprosterol, $C_{27}H_{48}O$.

Coprosterol has been found in human fæces; it is probably formed by the reduction of cholesterol in the large intestine.

Hippocoprosterol.

Two compounds, $C_{27}H_{54}O$ and $C_{27}H_{52}O$, have been isolated from horses' manure and are probably reduction products of phytosterol.

Phytosterols, $C_{27}H_{46}O$.

Compounds very similar to cholesterol have been prepared from plants and have been termed phytosterols. They are probably mixtures of isomeric compounds, that from the Calabar bean having been shown to be a mixture of sitosterol, $C_{27}H_{45}OH$, and stigmasterol, $C_{30}H_{47}OH$. They are mostly contained in the fat of plant seeds.

They are prepared from the vegetable fat of the seeds by saponification with alcoholic potash; the alcohol is evaporated and the residue dissolved in water. The aqueous solution is extracted with ether, and this extract, on evaporation, yields the phytosterol, which is recrystallised from alcohol.

The phytosterols crystallise like cholesterol, but the crystals are usually six-sided. The melting-point of the phytosterols varies from $135\text{-}144°$, but is usually $135\text{-}137°$. In solubility they resemble cholesterol and form acetates and benzoates. The acetates melt at $125\text{-}137°$. They can only be distinguished from cholesterol by their crystalline form and the melting-point of the acetates.

BILE ACIDS.

The bile of animals contains the sodium salts of glycocholic and taurocholic acids, glycocholeic and taurocholeic acids. These acids are decomposed by boiling the bile with sodium hydroxide and yield glycine or taurine, and cholalic or choleic acids.

Cholalic Acid, or Cholic Acid, $C_{24}H_{40}O_5$.

$$C_{20}H_{31}\begin{cases} COOH \\ CHOH \\ CH_2OH \\ CH_2OH \end{cases}$$
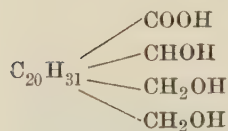
Though the constitution of cholalic acid is unknown, its properties and reactions show that it should be included amongst the hydro-aromatic compounds. It has a structure very similar to that of cholesterol. Its products of oxidation and their properties show that it is a trihydroxy monobasic acid.

Glycocholic Acid, $C_{26}H_{43}O_6N$.

$$C_{23}H_{39}O_3 . CO$$
$$| \qquad\qquad NH\text{---}CH_2 . COOH.$$
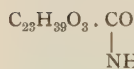
Glycocholic acid is present in ox and human bile (7-9 per cent.), but not in that of carnivora. It is converted by hydrolysis with acids into cholalic acid and glycine. Its constitution is similar to that of hippuric acid.

Preparation.   Hüfner's Method.

Fresh ox bile is covered with a layer of ether in a measuring cylinder and

treated with concentrated hydrochloric acid (2 c.c. to 40 c.c. bile). The turbidity at first formed becomes crystalline. The ether is poured off, the mass stirred with water, well shaken, filtered and washed with cold water till the washings are colourless. The precipitate contains glycocholic acid and glycocholeic acid.

Properties.

Glycocholic acid crystallises in needles (Fig. 46) which are soluble with difficulty in cold water (1 part in 300), more easily in hot water (1 part in 120), very easily soluble in alcohol and in alkalies, almost insoluble in ether. The alcoholic solution, on the
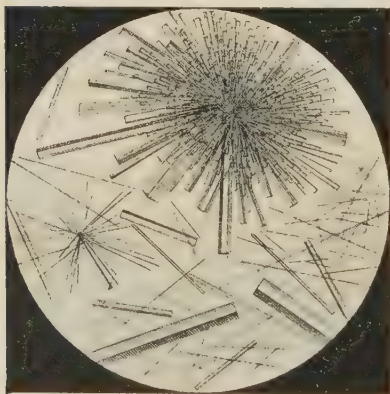
FIG. 46.—Glycocholic acid. (After Funke.)

addition of water, becomes turbid and deposits crystals. The alkaline solution is precipitated by acids. On heating in a capillary tube, glycocholic

acid softens at 133° and melts at 152°. It has a sweet-bitter taste and is dextrorotatory. The alkaline salts are obtained by evaporation of the alcoholic solution; they dissolve fats and cholesterol and give precipitates with lead acetate, ferric chloride, silver nitrate. The lead salt is soluble in hot alcohol and separates as a powder on cooling. The barium salt is easily soluble in water.

Taurocholic Acid, $C_{26}H_{45}O_7NS$.

$C_{23}H_{39}O_3 . CO$
   |
 $HN . CH_2$
   |
  $CH_2 . SO_3H.$
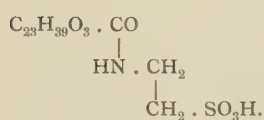
Taurocholic acid accompanies glycocholic acid in ox bile. It is present in fish bile and snake's bile. Dog's bile and the bile of carnivora do not contain glycocholic acid. In all cases it is present as the sodium salt. On hydrolysis, it is converted into taurine and of cholalic acid.

Reactions of Bile Acids.

The bile acids are distinguished by two characteristic reactions (1) Pettenkofer's reaction, (2) Hay's surface tension reaction. Their presence in bile (see p. 462) is shown by these reactions.

CHAPTER XXXVIII.

INDOLE AND ITS DERIVATIVES.

INDIGO, tryptophan, scatole, indole are natural substances which contain the heterocyclic indole ring—the complex nucleus made up of a benzene ring and a pyrrole ring :—

The compounds containing this ring have the properties of benzene and of pyrrole.

Indole.

Indole was first obtained by the reduction of indigo by distillation with zinc dust and also in the same way from other products obtained from indigo. It was identified as one of the products of the putrefaction of protein, together with scatole, and it is present in animal excrement. It is a constituent of coal tar and is isolated from the fraction of basic character which distils between 240 and 260°.

Indole has been found in essential oil of jasmine. About 2·5 per cent. of the oil, extracted by means of fat from the picked flowers, consists of indole. It is remarkable that the oil from fresh flowers does not contain indole. This oil, however, yields indole on steam distillation. Other flowers have also been found to contain indole. Jasmine oil has thus become a convenient source of indole.

Its constitution has been shown to be :—
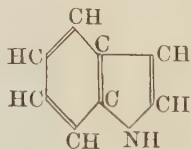
by various syntheses. A good yield is obtained on heating the hydrochloride of stilbene to 170° :—

$$C_6H_4\!\!\diagup\!\!\begin{array}{c} CH\!=\!CH\!-\!C_6H_4NH_2 \\ \\ NH_2 \end{array} = C_6H_4\!\!\diagup\!\!\begin{array}{c} CH \\ \diagdown CH \\ NH \end{array} + C_6H_5NH_2.$$

348
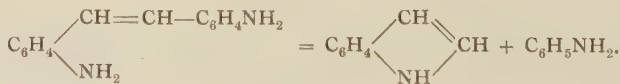
Properties.

Indole crystallises from petroleum ether in glistening platelets which melt at $52\cdot5°$. It is volatile in steam and these vapours have a peculiar and unpleasant smell. Pure indole has a pleasant smell. It is used to give freshness to perfumes. It is fairly soluble in hot water, alcohol, ether, chloroform, benzene, ligroin.

It is a weak base, a secondary amine, and combines with strong acids to form salts. Its picrate $C_8H_7N \cdot C_6H_3O_7N_3$, which forms long and shining needles, is useful for its identification.

Reactions.

* (1) A pine shaving moistened with hydrochloric acid and introduced into an alcoholic solution of indole becomes red.

* (2) On the addition of a few drops of nitric acid and, drop by drop, a few drops of very dilute potassium nitrite solution ($\cdot1$ per cent.) to a solution of indole, the solution becomes red and in strong solutions a precipitate of nitrosoindole is formed. (Baeyer.)

* (3) On adding half the volume of a 2 per cent. alcoholic solution of p-dimethylaminobenzaldehyde to a solution of indole and, drop by drop, 25 per cent. hydrochloric acid, a red colour appears. The subsequent addition of a few drops of $0\cdot5$ per cent. sodium nitrite solution gives a dark red colour which soon fades. (Ehrlich.)

* (4) On adding sodium nitroprusside solution to indole solution until it is of a yellow colour and then a few drops of caustic soda, a deep violet-blue colour is obtained; the addition of acetic acid changes it to pure blue. (Legal.)

* (5) If under a solution of indole treated with glyoxylic acid a layer of concentrated sulphuric acid be run, a red colour is produced at the point of contact. (Hopkins.) This reaction is sensitive to 1 in 500,000.

* (6) If formaldehyde be used instead of glyoxylic acid, a similar colour is produced. This reaction is sensitive to 1 in 700,000 (Kondo.)

Scatole, or β-Methyl Indole.
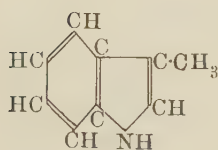
Scatole was first isolated from fæces (skatos) and recognised as a constituent of the intestinal contents of man and animals. Later, it was obtained by the fusion of proteins with alkali and isolated from the products of putrefaction. The secretion (civet) of the civet cats contains $0\cdot1$ per cent. of scatole. It is largely used in perfumery.

Scatole can be synthesised from propionic aldehyde phenylhydrazone by heating it with zinc chloride :—

$$C_6H_4 \begin{array}{c} {}^H \\ \diagup \\ \diagdown \\ NH-N \end{array} \begin{array}{c} CH_2-CH_3 \\ | \\ CH \\ | \end{array} = C_6H_4 \begin{array}{c} C.CH_3 \\ \diagup \parallel \\ \diagdown \diagup \\ NH \end{array} CH + NH_3$$
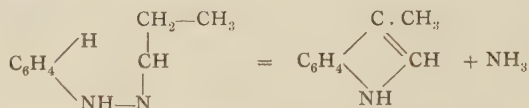
Scatole crystallises in colourless platelets melting at 95° and boiling at 265-266°. It has an intense fæcal smell. It dissolves in water, but less readily than indole, but is more easily volatile in steam than indole. It dissolves in alcohol, ether, benzene, chloroform.

It is a weak base and combines with acids to form salts; the hydrochloride is easily soluble in alcohol, but insoluble in water and ether.

Reactions.

(1) It dissolves in concentrated hydrochloric acid giving a violet coloured solution. A purple-red is formed on warming its solution in sulphuric acid.

* (2) With nitric acid and sodium nitrite, it gives a white turbidity (compare indole).

* (3) With p-dimethylaminobenzaldehyde solution, it gives a blue-violet colour which turns blue with sodium nitrite (compare indole).

* (4) With sodium nitroprusside and soda, it gives a yellow colour, which turns violet on heating for a few minutes with half its volume of glacial acetic acid.

* (5) The glyoxylic acid reaction is rose-red in colour.

* (6) The formaldehyde reaction is yellow or brown, but red if a trace of a ferric salt be present.

(7) The pine shaving reaction is negative, but if a pine shaving dipped in an alcoholic solution of scatole be placed in cold concentrated hydrochloric acid it becomes cherry-red, changing after a little while to a dark violet.

Indoxyl and Indican.
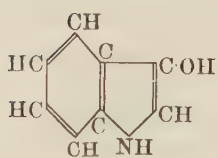
Indoxyl occurs in various species of the indigofera and in woad, *Isatis tinctoria*, in combination with glucose as the glucoside, indican. The glucoside is hydrolysed by enzymes in the plant leaves and converted into indoxyl, which undergoes oxidation to indigo blue.

Indoxyl occurs in human and mammalin urine in combination with

sulphuric acid (and glycuronic acid) as ester and is also known as indican. Indican is hydrolysed by acid and oxidised to indigo blue.

Indoxyl consists of yellow crystals which dissolve in water with a green fluorescence, also in alcohol, ether, acetone. It melts at 85°. Dilute acids convert it into a red substance and an unpleasant smell is produced. In alkaline solution, it oxidises in the air to indigo.

Detection in Urine.

Indoxyl is detected by conversion into indigo blue :—

(1) An equal volume of concentrated hydrochloric acid is added to 10 c.c. of urine and 2 or 3 c.c. of chloroform. A very dilute solu-tion of bleaching powder is added, *drop by drop*, and the solution is inverted after the addition of each drop. The chloroform becomes bluish-violet owing to the formation of indigo blue. Excess of bleach-ing powder must be avoided, as the indigo blue undergoes further oxi-dation to colourless compounds.

(2) The further oxidation is to a large extent avoided by using a fresh solution of ferric chloride in concentrated hydrochloric acid as the oxidising agent (Obermayer's reagent). An equal volume of this reagent and a few c.c. of chloroform are added to the urine. On mixing thoroughly for 1-2 minutes by inverting the liquids, the chloroform becomes blue.

(3) Salkowski recommends the use of copper sulphate as the oxidising agent to prevent further oxidation. An equal volume of hydrochloric acid, 1 c.c. of copper sulphate solution and a few c.c. of chloroform are added to 10 c.c. of urine and the mixture shaken carefully as above.

(4) The following other oxidising agents may be employed :—

One drop of a 10 per cent. solution of potassium persulphate to 5 or 6 c.c. of urine.

One drop of a 3 per cent. solution of potassium chlorate to 10 c.c. of urine.

Indigo Blue, or Indigotin.

Indigo blue is formed by the oxidation of indoxyl in alkaline solu-tion on exposure to the air. Two molecules of indoxyl combine in this reaction :—
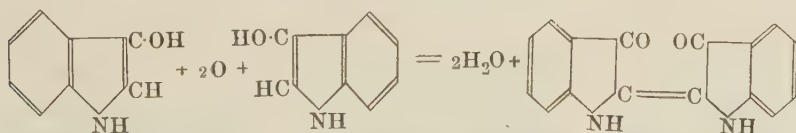
Indigo blue is a dark blue powder and shows a metallic coppery lustre on rubbing. It sublimes giving copper-red glistening prisms. It is insoluble in water, alcohol, ether, dilute acids and alkalies, and has

neither smell nor taste. It dissolves in aniline and molten paraffin with a purple-red colour, also in turpentine from which it crystallises.

Owing to its insolubility it is converted into indigo white, or into indigotin sulphonic acid, so as to be used as a dye.

Indigo blue has been synthesised by various methods. The synthetic product is cheaper than the natural and is gradually displacing the natural product as a dye.

Indigo White.
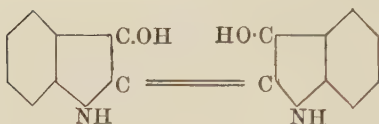
The insolubility of indigo blue renders it useless as such for dyeing purposes. On reduction by zinc dust and alkali, hydrosulphite, or by electrolysis, it is converted into indigo white. In air, this solution reoxidises and forms indigo blue.

In dyeing, the indigo blue is reduced, the cloth soaked in the solution and exposed to the air. Insoluble indigo blue is deposited on the fibres.

Indigo white can be precipitated from solution in absence of air as white crystals which dissolve in alcohol, ether and alkalies with a yellow colour.

Indirubin, or Indigo Red, and Isoindigotin.

Natural indigo blue is generally associated with small quantities of indigo red. This is formed by a combination of 1 molecule of indoxyl with 1 molecule of the isomeric oxindole :—
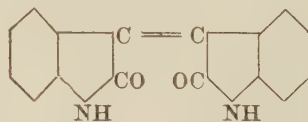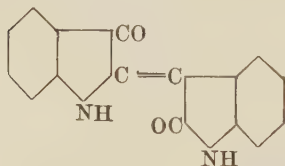
Combination occurs between the a and β carbon atoms. If combination occurs between the two β carbon atoms, isoindigotin is formed.

Indole-β-Acetic Acid.
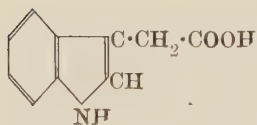
This compound was first found amongst the putrefactive decomposition products of proteins. It is often present in urine and is found particularly in cases of intestinal disorder.

It crystallises in platelets melting at 164° and is soluble with difficulty in water, but easily in alcohol and ether. On heating, it decomposes into carbon dioxide and scatole.

Reactions.

(1) On adding a few drops of pure nitric acid and, drop by drop, a 2 per cent. solution of potassium nitrite to a solution of indole-acetic acid, a cherry-red colour is formed, followed by a turbidity and separation of a red pigment.

(2) A purple-red colour and precipitate is formed when an equal volume of concentrated hydrochloric acid and a few drops of a 1-2 per cent. solution of bleaching powder are added to its solution.

(3) A violet colour is formed (before and after boiling), if an equal volume of concentrated hydrochloric acid and a few drops of ferric chloride solution be added to a solution of indole-acetic acid.

(4) A red colour is formed with p-dimethylaminobenzaldehyde (see under indole).

The Urorosein Reaction of Urine.

This reaction consists in the formation of a red pigment when concentrated hydrochloric acid and a drop or two of sodium nitrite solution is added to urine. Stale urines give this reaction on the addition of hydrochloric acid only, as nitrites are formed by bacterial decomposition of other constituents in the urine. The colour disappears on adding alkali, but reappears on acidifying.

Urorosein is insoluble in ether and chloroform, but dissolves in alcohol and amyl alcohol with a red colour. The amyl alcoholic solution shows an absorption band in the green, between D and E, but nearer D. Urorosein is most probably nitrosoindole-acetic acid.

Indole-β-Propionic Acid.
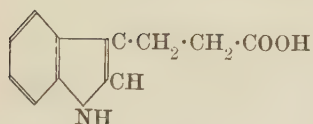
Indole propionic acid has also been shown to be a putrefactive decomposition product of proteins.

It crystallises in prisms, or irregular plates, is slightly soluble in water, but easily soluble in alcohol and ether.

Reactions.

(1) Indole-β-propionic acid forms a nitroso compound with potassium nitrite. In concentrated solution on the addition of concentrated potassium nitrite and acetic acid, a yellow crystalline mass may be obtained.

(2) An aqueous solution gives a white turbidity with ferric chloride which becomes red on heating.

Indole-ethylamine.
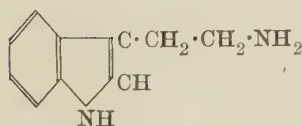
Indole-ethylamine is another substance which is formed in the putrefaction of proteins and of tryptophan. It is one of the amines which have a marked physiological action, but is not so marked in its action as p-hydroxy-phenyl-ethylamine, or iminazolyl-ethylamine.

23

Tryptophan.

C · CH$_2$ · CH(NH$_2$) · COOH

CH

NH
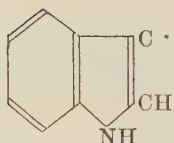
Tryptophan was shown to be a constituent of proteins by Hopkins and Cole in 1902, who isolated it from the mixture of amino acids which results from the digestion of proteins with the enzyme, trypsin.   Its discovery gave the clue to the well-known Adamkiewicz reaction of proteins and the proteinochrome reaction of tryptic digests.

Tryptophan is prepared by precipitating a trypsin digest of caseinogen (or other protein) with mercuric sulphate in 5 per cent. sulphuric acid solution.   The precipitate is filtered off, and decomposed with hydrogen sulphide.   After repeating the precipitation with mercuric sulphate and neutralising, tryptophan separates out on concentrating and is recrystallised from a mixture of alcohol and water.

Tryptophan crystallises in colourless glistening platelets which are not easily soluble in cold water, but readily in hot.   It is insoluble in absolute alcohol and ether.   It dissolves easily in hot pyridine, less easily in cold.   On heating in a capillary tube it changes colour at 220°, becomes brown at 240° and melts at 252°.

If heated quickly, it turns yellow at 260° and melts at 289°.   Tryptophan is a weak base and forms salts with acids.   As an amino acid, it forms salts and forms acyl derivatives with acid chlorides, some of which serve for its isolation and characterisation.

Reactions.

Tryptophan, even when mixed with other amino acids, is readily recognised in solution by the following reactions :—

* (1) *Bromine water reaction.*—About 5 c.c. of the solution are acidified with acetic acid and bromine water is added, drop by drop ; a reddish-violet colour appears.   This gradually deepens, but disappears if too much bromine water be added, giving a yellow solution. When the maximum reddish-violet colour is obtained, the solution is shaken with 2 or 3 c.c. of amyl alcohol ; the amyl alcohol dissolves the pigment and separates coloured reddish-violet.

* (2) *Glyoxylic acid reaction.*—A small quantity of glyoxylic acid solution is added to about 5 c.c. of the solution and concentrated sulphuric acid is run under its surface ; at the point of junction a reddish-violet ring appears and on gently mixing the two liquids the colour spreads throughout the mixture.

(3) On mixing a tryptophan solution with an alcoholic solution of benzaldehyde and running underneath it concentrated sulphuric acid containing ferric sulphate, a blue colour is formed at the junction.

(4) Using formaldehyde instead of benzaldehyde, the colour at the junction of the liquids is blue-violet.

(5) With p-dimethylaminobenzaldehyde and concentrated hydrochloric acid, a red colour is formed (see under indole).

(6) A pine shaving wetted with hydrochloric acid, washed with water, dipped into a concentrated solution of tryptophan and dried, becomes purple in colour.

The Biological Relationship of the Indole Derivatives.

Indole and scatole have long been known to be putrefactive products of protein, the other compounds were found later. The discovery of tryptophan and the determination of its constitution has shown that all these compounds are derived from it; in many cases the direct conversion of tryptophan in putrefaction has been carried out. The stages in the decomposition of tryptophan are similar to those which tyrosine undergoes, namely :—

Indole ethylamine.   Tryptophan.   Indole propionic acid.

Indole acetic acid.   Scatole.   Indole.
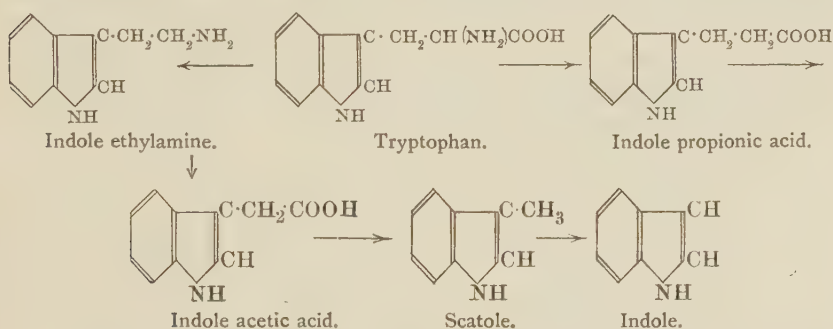
Indole is oxidised and converted into indoxyl, which is combined with sulphuric acid (or glucose) to form indican. Indican on hydrolysis yields indoxyl, which is oxidised in the air, or by oxidising agents, to indigo blue :—

Indican.   Indoxyl.   Indigo blue.

23 *
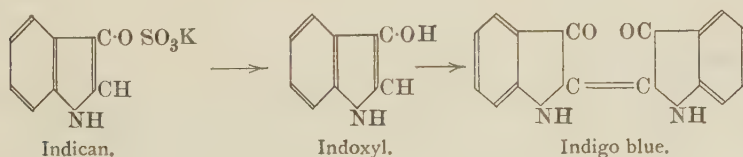
CHAPTER XXXIX.

THE ALKALOIDS.

THE term vegetable alkaloids was originally applied to the group of basic substances (hence the name alkaloid), which were found in plants, to distinguish them from basic substances (amines, formerly ptomaines or toxines) found in animals, formed mainly by putrefaction. The term alkaloid is now applied only to the basic substances occurring in plants which contain in their constitution either a pyridine, quinoline, iso-quinoline, or pyrrole, or pyrrolidine, ring, or several rings. They are classified according to the ring into the following groups :—

 I. Pyridine group :—

   Piperine, coniine, trigonelline nicotine.

 II. Pyrrolidine group :—

   Hygrine, stachydrine.

 III. Tropane group :—

   Atropine, hyoscyamine, hyoscine, cocaine.

 IV. Quinoline group :—

   Quinine, cinchonine, strychnine, brucine.

 V. Isoquinoline group :—

   Narcotine, narceine, morphine, codeine, papaverine, berberine.

 The constitution of most of the alkaloids is known, but some are still under investigation. The details of the work upon the elucidation of their constitution are very complex. Only the formulæ of the chief compounds can therefore be given so as to show their relationship to pyridine and the other nuclei.

 The alkaloids generally occur in plants in the form of salts with organic acids, such as citric, tartaric, malic, oxalic, succinic. They are liberated from the salts by means of alkali and can frequently be extracted from the alkaline solution by means of chloroform, ether, and other organic solvents. Most of the alkaloids are solid compounds ; coniine, nicotine, and a few others are liquid. They generally contain oxygen in their composition, but again there are exceptions. Most alkaloids give precipitates with the following reagents :—

(1) tannic acid ;
(2) picric acid ;
(3) iodine in potassium iodide ;
(4) mercuric iodide in potassium iodide ;
(5) phosphotungstic acid and phosphomolybdic acid.

These reagents, termed alkaloidal reagents, also give precipitates with amines and other bases and with proteins. They cannot in consequence be considered as specific for the alkaloids, but they are sometimes useful for isolating and detecting alkaloids.

Piperine.

Piperine is found in the fruit of the various varieties of pepper; about 8 or 9 per cent. is present in black pepper.

Powdered pepper is warmed with lime water for 15-20 minutes and the mixture is evaporated to dryness. The dry residue is extracted with ether. The ethereal solution on distillation leaves piperine, which is purified by crystallisation from alcohol.

Piperine is a white solid which melts at 128°. It is almost insoluble in water. It behaves as a weak base and dissolves in concentrated sulphuric acid giving a deep red solution.

On distillation with alcoholic potash, piperine is converted into piperidine and piperic acid. Piperine is the amide of piperidine and piperic acid. It has been synthesised by the action of piperic acid chloride on piperidine.

Piperine.

Piperidine.

Piperic acid, or
3, 4 methylene dihydroxycinnamenyl.
acrylic acid
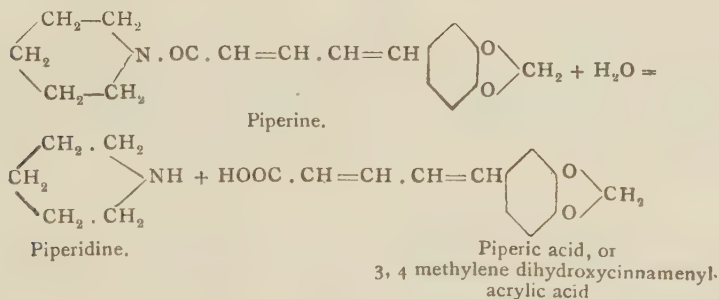
Coniine, $C_8H_{17}N$.

Coniine is the alkaloid which is present in the seeds of hemlock. It is prepared therefrom by distillation with sodium hydroxide.

Coniine is a colourless liquid which boils at 167°. It has a peculiar and penetrating odour and turns brown in the air. It is soluble in water and is a strong base forming salts with acids.

The natural substance is optically active and dextrorotatory.

Coniine has been shown to be a-propylpiperidine and has been synthesised as follows :—

$$\text{Pyridine.} + CH_3I = \quad \underset{CH_3\ I}{N} \quad \xrightarrow{\text{heat}} \quad \underset{\text{α-picoline}}{CH_3}$$

$$CH_3 + OHC . CH_3 = \quad CH{=}CH . CH_3 + H_2O$$

$$\text{Acetaldehyde.} \qquad \text{α-allyl-pyridine.}$$

$$CH{=}CH . CH_3 + 3H_2 = \underset{\text{α-propylpiperidine.}}{H_2C \genfrac{}{}{0pt}{}{CH_2}{} CH . CH_2 . CH_2 . CH_3}$$
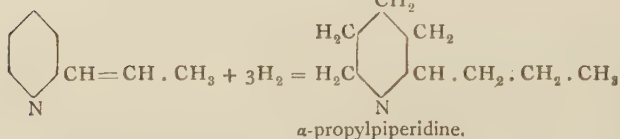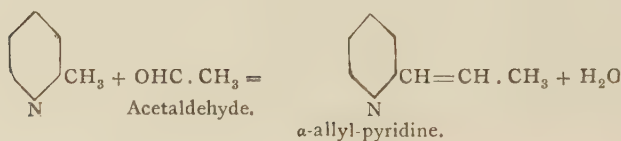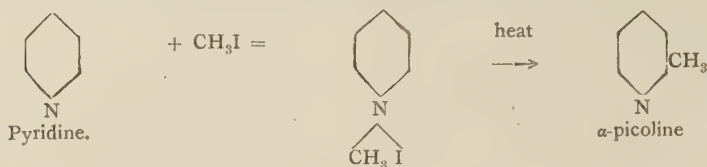
The inactive coniine was separated into *d*- and *l*-coniine by the fractional crystallisation of its tartrate.

Nicotine, $C_{10}H_{14}N_2$.

Nicotine occurs to the extent of ·6-8 per cent. in tobacco leaves in combination with organic acids (malic or citric).

Tobacco leaves are boiled out with water. The aqueous solution is concentrated, made alkaline with lime and distilled. The distillate is acidified with oxalic acid and evaporated. The concentrated solution is rendered alkaline with soda and extracted with ether. The ethereal extract on distillation leaves the nicotine, which is purified by distillation in a current of hydrogen.

Nicotine is a colourless oily liquid which boils at 241°. It possesses an unpleasant smell and a burning taste. It is intensely poisonous. In air it undergoes oxidation, turning brown.

It is a ditertiary base and forms salts with acids, which are dextrorotatory, and combines with 2 molecules of methyl iodide.

On oxidation with chromic acid, it yields nicotinic acid showing that it possesses a substituting group in the β-position of the pyridine ring. This substituting group has been found to be N-methyl-pyrrolidine, the methyl group being attached to the nitrogen atom. It is β-N-methyl-pyrrolidine-pyridine.
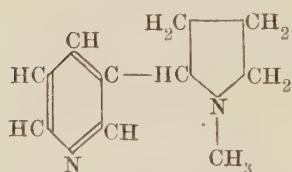
Hygrine.

Hygrine has been shown to be β-acetyl-N-methyl-pyrrolidine.

$$\begin{array}{c} H_2C \text{———} CH\text{-}CO\text{·}CH_3 \\ H_2C \diagdown \quad \diagup CH_2 \\ N \\ | \\ CH_3 \end{array}$$
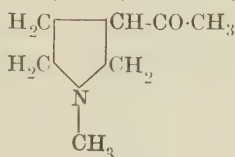
Atropine, or dl-Hyoscyamine, $C_{17}H_{23}O_3N$.

Atropine (or daturin) is found in the deadly nightshade, *Atropa belladonna*, in henbane, *Hyoscyamus niger*, and together with *l*-hyoscyamine in *Datura stramonium*.

The juice (1 litre) obtained by pressing the plant is made alkaline with potash (4 gm.) and extracted with chloroform (25 c.c.). The chloroform extract is evaporated and the residue is treated with dilute sulphuric acid which dissolves the base. On adding potassium carbonate to the acid solution, the atropine is precipitated and is crystallised from alcohol.

Atropine is a white crystalline solid separating in prisms from dilute alcohol. It melts at 115°, is almost insoluble in water but is easily soluble in alcohol, ether, and chloroform. It is extremely poisonous, from ·05-·2 gm. being a lethal dose. It is a strong base and forms salts with acids which are easily soluble in water. The sulphate is most commonly used in medicine for dilating the pupils and other purposes.

Atropine may be tested for as follows: on evaporating a small quantity with a drop of fuming nitric acid, a yellow residue is left. This turns violet, changing to red, on adding a drop of alcoholic potash.

On hydrolysis by boiling with baryta, atropine is converted into tropine and tropic acid.

Tropic acid is a-phenyl-β-hydroxypropionic acid.

Tropine has been shown to be the N-methyl derivative of a γ-hydroxy-piperidine nucleus containing two extra methylene groups, or as a hydroxy derivative of a combined piperidine and pyrrolidine nucleus to which a methyl group is attached at the nitrogen atom.

$$\begin{array}{c} CHOH \\ H_2C \diagup \quad \diagdown CH_2 \\ | \quad H_2 \quad H_2 \quad | \\ HC \diagdown C\text{—}C \diagup CH \\ N \\ | \\ CH_3 \end{array}$$

Atropine is the tropic acid ester of tropine :—

$$\begin{array}{c} C_6H_5 \\ | \\ CH\text{·}O\text{·}OC\text{·}CH\text{·}CH_2OH \\ H_2C \diagup \quad \diagdown CH_2 \\ | \quad H_2 \quad H_2 \quad | \\ HC \diagdown C\text{—}C \diagup CH \\ N \\ | \\ CH_3 \end{array}$$
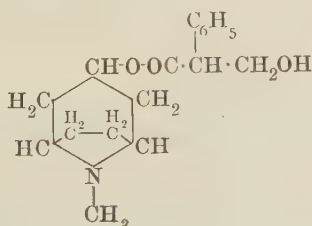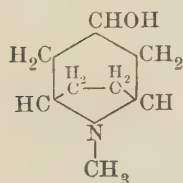
and it has been synthesised from its constituents.

Cocaine, $C_{17}H_{21}O_4N$.

Cocaine is found in the leaves of coca, *Erythroxylon Coca*, from which it is prepared as follows :—

The leaves are treated with hot water at a temperature of about 80°. The filtered solution is precipitated with lead acetate to remove tannins, proteins, etc. The excess of lead is removed from the filtrate by adding sodium sulphate, and the solution, after again filtering, is made alkaline with soda and extracted with ether. The residue obtained on evaporation of the ether is crystallised from alcohol.

Cocaine separates out in colourless prisms which melt at 98°. It is not easily soluble in water, but is soluble in organic solvents. It is a strong base forming salts, the hydrochloride being the salt most frequently used in medicine. Its use in medicine depends upon its being a local anæsthetic.

On hydrolysis by acids, cocaine is converted into methyl alcohol, benzoic acid, and ecgonine.

Ecgonine has been found to be closely related to tropine and is a carboxylic acid of tropine :—

$$
\begin{array}{c}
CHOH \\
H_2C \quad\quad CH\cdot COOH \\
\quad H_2\ H_2 \\
HC \quad C-C \quad CH \\
N \\
| \\
CH_3
\end{array}
$$
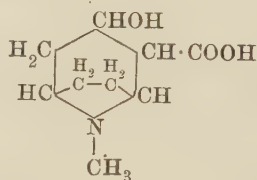
Cocaine is the double ester of ecgonine with benzoic acid and methyl alcohol and has the following constitution :—

$$
\begin{array}{c}
CH.O\cdot OC\cdot C_6H_5 \\
H_2C \quad\quad CH\cdot COOCH_3 \\
\quad H_2\ H_2 \\
HC \quad C-C \quad CH \\
N \\
| \\
CH_3
\end{array}
$$
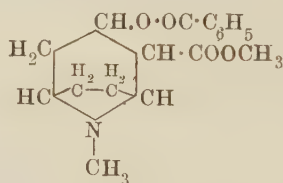
This formula has been proved by the synthesis of cocaine from these constituents.

Other esters of ecgonine have been prepared using different acids in the place of benzoic acid and different alcohols in the place of methyl alcohol.

Cinchonine, $C_{19}H_{22}ON_2$.

Cinchonine is present, together with quinine, in the varieties of cinchona bark, the grey bark containing as much as 2·5 per cent.

It is prepared from the solutions remaining from the preparation of quinine ; they are made alkaline with caustic soda and the precipitate so formed is

dissolved in a small quantity of boiling alcohol; on cooling, cinchonine is deposited. It is purified by converting it into its sulphate and crystallising from water.

Cinchonine separates in colourless prisms which melt at 255°, is soluble with difficulty in water, but more easily soluble in alcohol, ether, etc. It is a weak base and it forms salts with acids. It is a tertiary base, combining with 2 molecules of methyl iodide.

Cinchonine is an unsaturated compound and combines with 2 atoms of bromine, or with 1 molecule of hydrochloric acid.

On oxidation, it yields cinchonic acid, or quinoline-γ-carboxylic acid, and another product which has been shown to be a piperidine derivative.

Cinchonine is thus a derivative of quinoline and contains a piperidine ring. Its constitution is the following :—
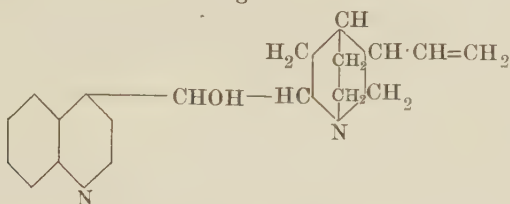
Quinine, $C_{20}H_{24}N_2O_2$.

Quinine, together with cinchonine, is contained in cinchona bark up to about 3 per cent.; the yellow bark of Calisaya contains up to 12 per cent.

The bark is powdered and treated with dilute sulphuric acid; from the acid solution the bases are precipitated by adding soda. The mixture of bases is dissolved in alcohol and the solution is neutralised with sulphuric acid. The sulphates, which are so obtained, are recrystallised from water. Quinine sulphate is the most insoluble and separates out first. It is converted into quinine by precipitation with ammonia.

Quinine crystallises from water with 3 molecules of water of crystallisation. The anhydrous substance melts at 173°. It is very slightly soluble in water, but is soluble in alcohol and ether. It has a bitter taste and is a feeble base. It forms salts with acids. The sulphate and hydrochloride are used in medicine. Like cinchonine, it is a ditertiary base and combines with 2 molecules of methyl iodide.

Quinine may be tested for by the following reaction :—

A solution of a quinine salt on the addition of chlorine water, or bromine water, followed by ammonia, gives a green precipitate. This dissolves in excess of ammonia giving an emerald-green solution.

Quinine is very similar in constitution to cinchonine and is methoxy-cinchonine :—
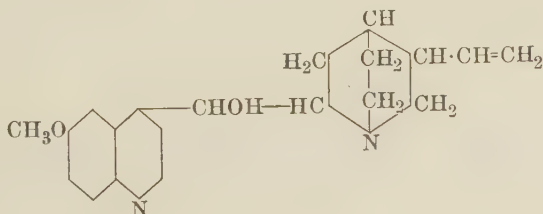
Strychnine, $C_{21}H_{22}O_2N_2$.

Strychnine is the poisonous alkaloid of the seeds of *Strychnos nux vomica*, in which it is present together with brucine.

The powdered seed is extracted with hot dilute alcohol.   The alcoholic solution is evaporated and the aqueous remainder is treated with lead acetate which precipitates tannin, proteins, etc.   The excess of lead is removed by treatment with hydrogen sulphide and the filtrate from the lead sulphide is freed from hydrogen sulphide.   Magnesia is added to precipitate the alkaloids which are filtered off after standing.   The mixture of brucine and strychnine is separated by alcohol which dissolves the brucine.   The strychnine is purified by crystallisation from alcohol.

Strychnine crystallises in colourless prisms which melt at 284°.   It is very slightly soluble in water and its solutions have a very bitter taste.   It is a weak base and forms salts with acids.

Though it contains 2 nitrogen atoms it is only a monacid base and combines with only 1 molecule of methyl iodide.

Strychnine gives the following reaction by which it may be detected :—

On treating a small quantity of strychnine, or a strychnine salt, with concentrated sulphuric acid in a porcelain basin and on adding a small amount of powdered potassium bichromate, a violet solution is produced which becomes red and then yellow.

The constitution of strychnine has not yet been definitely determined, but it is a derivative of quinoline.

Brucine, $C_{21}H_{20}(OCH_3)_2O_2N_2$.

Brucine is present with strychnine in the seeds of nux vomica.

The alcoholic solution, in which the brucine has dissolved in the separation from strychnine, is evaporated to dryness.   The residue is dissolved in dilute acetic acid and this solution is evaporated so as to remove the strychnine which is also contained in it.   The strychnine separates out on evaporation and is filtered off, the strychnine acetate being an unstable salt.

The brucine acetate is dissolved in water and the brucine is precipitated by adding caustic soda.   It is crystallised from alcohol.

Brucine crystallises from water in colourless prisms with 4 molecules of water of crystallisation.   The anhydrous substance melts at 178°.   It is slightly soluble in water and alcohol and is very similar to strychnine, but is not so poisonous.

It gives the following reaction :—

A deep brown-red colour is formed on adding nitric acid to a brucine salt; the colour changes to yellow on warming.   The colour becomes violet on adding stannous chloride.

Brucine seems to be a dimethoxy derivative of strychnine and is a derivative of quinoline.

Morphine, $C_{17}H_{19}NO_3$.

Morphine is the chief alkaloid contained in the heads of poppies, *Papaver somniferum.*   The alkaloids are present as sulphates and meconates.

Incisions are made in poppy heads and the juice which exudes is collected and dried. This dry mass is termed opium.

The opium is treated with boiling water and the solution containing the salts of the bases is made alkaline with milk of lime. Calcium meconate and the alkaloids are precipitated. The alkaline solution containing the morphine is concentrated and warmed with ammonium chloride, so as to form calcium chloride, as long as ammonia is evolved. On standing, morphine separates out and is crystallised from alcohol.

Morphine crystallises from alcohol in small colourless prisms with 1 molecule of water. It is slightly soluble in water and its solution has a bitter taste. It is soluble in alcohol. An alcoholic solution of opium is termed laudanum.

It is a base which forms salts with acids, the hydrochloride being used in medicine; it combines with 1 molecule of methyl iodide.

Morphine may be detected by the following reactions :—

(1) A deep blue coloration is formed on adding ferric chloride to a solution of a morphine salt.

(2) On adding a little morphine solution to iodic acid solution, iodine is liberated and forms a brownish-red precipitate which reacts with starch.

(3) On dissolving morphine in concentrated sulphuric acid and adding concentrated nitric acid, after about 15 hours a deep blue-violet colour, which changes to red, is produced.

It is converted into apomorphine by loss of 1 molecule of water on heating with concentrated hydrochloric acid.

Morphine contains one hydroxyl group and one alcoholic group and on distillation yields pyridine and quinoline. Its constitution has not yet been definitely determined, but is probably
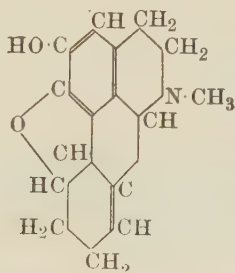
It thus contains an isoquinoline nucleus and a phenanthrene nucleus.

Codeine, $C_{17}H_{17}NO(OCH_3)OH.$

Codeine is also contained in opium. It can be obtained by methylating opium and is thus a methyl derivative of morphine. Its constitution is still not definitely known.

Papaverine, Laudanosine, Narcotine, Narceine.

These alkaloids are present in small quantities in opium with morphine. They are isoquinoline derivatives :—

24*
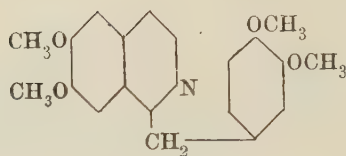
Papaverine,
or
tetramethoxybenzylisoquinoline.
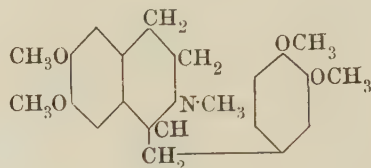
Laudanosine,
or
N-methyl-tetrahydropapaverine.
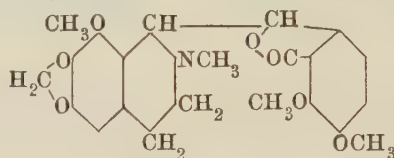
Narcotine.
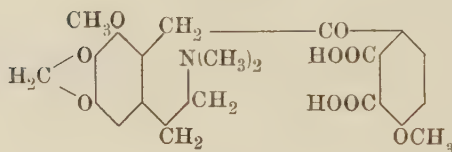
Narceine.

Narcotine yields cotarnine and meconin on hydrolysis :—
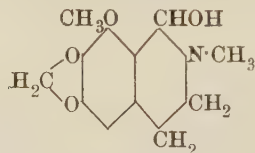
Cotarnine.
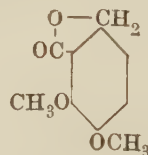
Meconin.

Berberine, $C_{20}H_{19}O_5N$.

Berberine is an alkaloid found in *Berberis vulgaris* and in other plants. It has a still more complicated structure :—
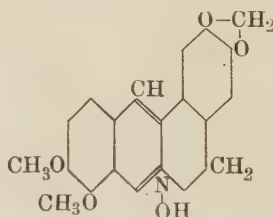
The details of the determination of the structure of the alkaloids can be found in the special books dealing with alkaloids.

CHAPTER XL.

THE PROTEINS.

PROTEINS make up the greater part of the solid matter of all animal cells and tissues and are present in various parts of plants. Meat and eggs consist mainly of protein; milk, seeds, and some fruits contain a large proportion of it. Protein is thus an essential ingredient of our food.

The proteins of the various tissues possess amongst themselves great physical differences, but have the very similar percentage composition of

$$C \quad 51\text{-}55 \text{ per cent.}$$
$$H \quad \quad 7 \quad ,, \quad \quad ,,$$
$$N \quad 15\text{-}17 \quad ,, \quad \quad ,,$$
$$S \quad 0{\cdot}4\text{-}2{\cdot}5 \quad ,, \quad \quad ,,$$
$$O \quad 20\text{-}30 \quad ,, \quad \quad ,,$$

and in some cases also $\quad P \quad 0{\cdot}1\text{-}1{\cdot}0 \quad ,, \quad \quad ,,$

Proteins are compounds of high molecular weight. Those which dissolve in water, or dilute salt solutions, show the properties of colloids (p. 381). Though generally amorphous, many have been prepared in a crystalline state. Hæmoglobin, which forms the red colouring matter of the blood is the most easily crystallised. It consists of hæmatin united with the protein, globin. Elementary analysis of the hæmoglobin of dog's blood gave

$$C \quad 53{\cdot}91 \text{ per cent.}$$
$$H \quad 6{\cdot}62 \quad ,, \quad \quad ,,$$
$$N \quad 15{\cdot}98 \quad ,, \quad \quad ,,$$
$$S \quad \quad 0{\cdot}54 \quad ,, \quad \quad ,,$$
$$Fe \quad 0{\cdot}33 \quad ,, \quad \quad ,,$$
$$O \quad 22{\cdot}62 \quad ,, \quad \quad ,,$$

These figures give the empirical formula of

$$C_{758}H_{1203}N_{195}S_3FeO_{218}.$$
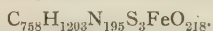
Deducting the empirical formula of $C_{33}H_{32}O_4N_4Fe$ of hæmatin, the empirical formula of the protein is

$$C_{725}H_{1174}N_{194}S_3O_{214}.$$
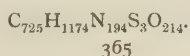
This formula is based on the presence of only 1 atom of iron in the molecule of hæmoglobin.   This formula gives a molecular weight of 16,110.   Similar data are found by the method of the depression of the freezing-point.   These are the lowest molecular weights and in many cases the molecular weight of a protein may be over 30,000.

Classification.

The known proteins are classified according to their origin, solubility, coagulability on heating and other physical properties without reference to their chemical composition.   This classification is, however, borne out by their actual chemical composition as far as it is known.

The following is the classification adopted by the Chemical and Physiological Societies in 1907 :—

    1. Protamines.

    2. Histones.

    3. Albumins ⎫

               ⎬ Coagulable Proteins.

    4. Globulins ⎭

    5. Glutelins.

    6. Gliadins, or Prolamins.[1]

    7. Scleroproteins.

    8. Phosphoproteins.

    9. Conjugated Proteins.

        (*a*) Nucleoproteins.

        (*b*) Glucoproteins.

        (*c*) Chromoproteins.

    10. Derivatives of Proteins.

        (*a*) Metaproteins.

        (*b*) Proteoses.

        (*c*) Peptones.

        (*d*) Polypeptides.

The properties of the different proteins are described in later chapters.

Composition of Proteins.

Proteins are composed of amino acids, which may be regarded as the units of the protein molecule just in the same way as a polysaccharide is composed of monosaccharide units and a fat of glycerol and various fatty acid units.

---

[1] Prolamins is the American terminology of this group.

The amino acids are obtained by the hydrolysis of proteins with acids, alkalies, or enzymes. Up to the present time 18 amino acids have been found to occur in the protein molecule –hence its complexity —but, though 18 units may be present in some proteins, others contain fewer, and in some cases a protein has been found to be composed of only 2 or 3 units. The percentage amounts of the various amino acids, which have been obtained by the hydrolysis of some of the proteins, are given in the following table :—

	Sturine.	Ox Muscle Protein.	Lactalbumin.	Gelatin.	Caseinogen.	Wheat Gliadin.	Wheat Glutenin.	Maize Zein.	Silk Fibroin.	Globin of Hæmoglobin.
Glycine		2·1	0	25·5	0	0	0·9	0	36·0	
Alanine		3·7	2·5	8·7	1·5	2·0	4·7	9·8	21·0	4·2
Valine		0·8	0·9	0	7·2	3·4	0·2	1·9		
Leucine		11·7	19·4	7·1	9·4	6·6	6·0	25·0	1·5	29·0
Isoleucine				0				—		
Aspartic acid		4·5	1·0	3·4	4·1	0·6	0·9	1·8	+	4·4
Glutamic acid		15·5	10·1	5·8	21·6	43·7	23·4	31·3	0	1·7
Serine				0·4	0·5	0·2	0·7	1·0	1·6	0·6
Hydroxyglutamic acid				0	10·5			2·5		
Proline		5·8	4·0	9·5	8·0	13·2	4·2	9·0	+	2·3
Hydroxyproline				14·1	0·3			—		1·0
Phenylalanine		3·2	2·4	1·4	3·2	2·4	2·0	7·6	1·5	4·2
Tyrosine		2·2	0·9	0·01	4·5	1·2	4·3	5·2	10·5	1·3
Tryptophan		+		0	1·7	1·0	+	0		+
Lysine	12·0	7·6	9·2	5·9	6·0	0·2	1·9	0	+	4·3
Arginine	58·2	7·5	3·2	8·2	3·8	3·2	4·7	1·8	1·0	5·4
Histidine	12·9	1·8	2·1	0·9	2·5	0·6	1·8	0·8	+	11·0
Cystine				?		0·5	0·02			0·3
Ammonia		1·1	1·3	0·4	1·6	5·2	4·0	3·6		
Total	83·1	67·5	57·0	91·31	86·4	83·0	59·72	101·5	73·1	69·7

We may notice in particular that sturine contains 58 per cent. of arginine, that hæmoglobin contains 11 per cent. of histidine, that silk-fibroin is composed chiefly of glycine, alanine, and tyrosine. Some vegetable proteins, those of the cereals, contain as much as 40 per cent. of glutamic acid. Numerous other differences can be noted, e.g. that glycine is present in globulins, but absent from albumins. No stress must be laid upon other small differences since the method of analysis is not a quantitative one. The amounts actually present are in most cases greater than is given. The total sum of the isolated amino acids is generally about 60 per cent. ; it is 70 per cent. in the case of hæmoglobin ; 90 per cent. has been obtained from gelatin and caseinogen. The best figures are those of zein.

The analytical data of all the proteins which have been analysed will be found in the "Chemical Constitution of the Proteins," Part I. In this book is also given a full account of the methods of analysis of the proteins. A brief outline of the method of separating certain amino acids has been given under the amino acids.

Constitution of the Proteins.   Polypeptides.

The work of Emil Fischer and his pupils has shown that the amino acid units are combined together in the form of acid amides, i.e. the carboxyl group of one amino acid is combined with the amino group of another amino acid, e.g. :

Glycyl-glycine   - $CH_2(NH_2) . CO—NH . CH_2 . COOH$
Alanyl-leucine   - $CH_3 . CH(NH_2) . CO—NH . CH(C_4H_9) . COOH$
Leucyl-alanine   - $C_4H_9 . CH(NH_2) . CO—NH . CH(CH_3) . COOH$
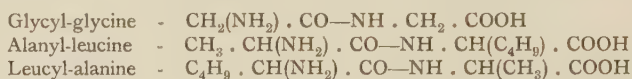
in which the units may either be the same, or different, and combined in any possible order.

These combinations of amino acids have been termed by Emil Fischer the polypeptides. The above compounds are dipeptides. In the same way we may have :—

Tripeptides, e.g.

Diglycyl-glycine, or glycyl-glycyl-glycine,
$CH_2(NH_2) . CO—NH . CH_2 . CO—NH . CH_2 . COOH.$
Glycyl-alanyl-tyrosine,
$CH_2(NH_2)CO—NH . (CHCH_3)CO—NH . CH(CH_2 . C_6H_4OH) . COOH).$
Alanyl-glycyl-tyrosine,
$CH_3 . CH(NH_2) . CO—NH . CH_2 . CO—NH . CH(CH_2 . C_6H_4OH) . COOH.$

Tetrapeptides, e.g.

Glycyl-alanyl-glycyl-tyrosine,
$CH_2(NH_2) . CO—NH . CH(CH_3)CO—NH . CH_2 . CO—NH . CH(CH_2 . C_6H_4OH) . COOH.$
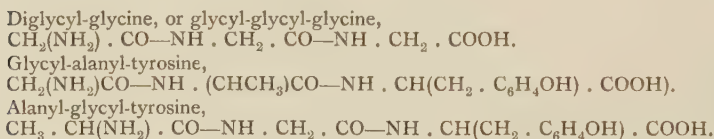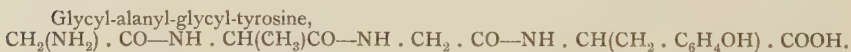
Pentapeptides, hexapeptides, etc.

The most complex polypeptide known is an octadecapeptide, which is composed of eighteen units made up of three leucine and fifteen glycine units. This compound, if it had been found in nature, would undoubtedly have been regarded as a true protein.

The synthesis of these polypeptides has been effected in three ways :—

(1) On heating the ester of an amino acid, a diketopiperazine is formed :—

$$
\begin{array}{c}
CH_2 . COOC_2H_5 \quad H_2N \\
| \qquad\qquad\qquad | \\
NH_2 \qquad C_2H_5OOC . CH_2
\end{array}
=
\begin{array}{c}
CH_2 . CO—HN \\
| \qquad\qquad | \\
NH——OC . CH_2
\end{array}
+ \ 2 C_2H_5OH.
$$
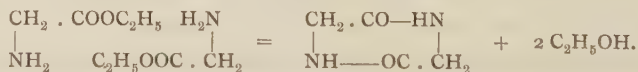
The diketopiperazine, on careful hydrolysis with dilute alkali, gives a dipeptide :—

$$
\begin{array}{c}
CH_2 . CO—HN \\
| \qquad\qquad | \\
NH——OC . CH_2
\end{array}
+ \ H_2O =
\begin{array}{c}
CH_2 . CO—HN \\
| \qquad\qquad | \\
NH_2 \qquad CH_2 . COOH.
\end{array}
$$
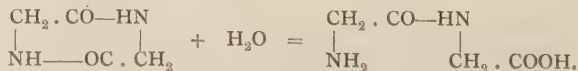
This method is mostly of historical interest.

(2) By treating an amino acid, or its ester, with the acid chloride of the corresponding halogen derivative of an amino acid, the halogen precursor is formed :—

$$
\underset{\substack{\text{Chloracetyl}\\\text{chloride.}}}{\overset{\displaystyle CH_2Cl}{\underset{\displaystyle CO.Cl}{|}}} + \underset{\substack{\text{Alanine.}}}{\overset{\displaystyle CH_3}{\underset{\displaystyle COOH}{\overset{|}{\underset{|}{CH(NH_2)}}}}} = \underset{\substack{\text{Chloracetyl alanine.}}}{\overset{\displaystyle CH_2Cl}{\underset{\displaystyle COOH}{\overset{|}{\underset{|}{CO—HN.CH.CH_3}}}}} + HCl
$$

$$
\underset{\substack{\text{Chlorpropionyl}\\\text{chloride.}}}{\overset{\displaystyle CH_3}{\underset{\displaystyle CO.Cl}{\overset{|}{\underset{|}{CHCl}}}}} + \underset{}{\overset{\displaystyle CH_3}{\underset{\displaystyle COOH}{\overset{|}{\underset{|}{CH.(NH_2)}}}}} = \underset{\substack{\text{Chlorpropionyl-}\\\text{alanine.}}}{\overset{\displaystyle CH_3}{\underset{\displaystyle COOH}{\overset{|}{\underset{|}{\overset{\displaystyle CH.Cl}{\underset{|}{\overset{CO—HN.CH.CH_3}{}}}}}}}} + HCl
$$
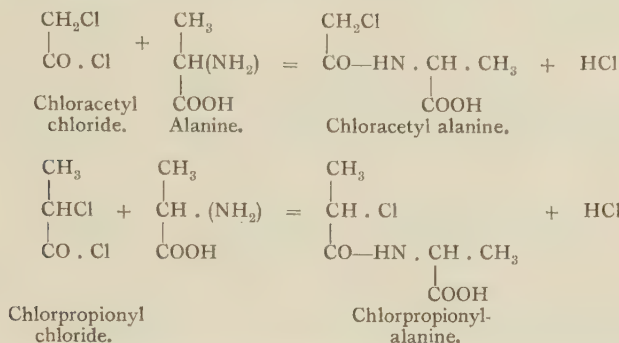
This compound gives the dipeptide on treatment with ammonia :—

$$
\underset{\substack{}}{\overset{\displaystyle CH_2Cl}{\underset{\displaystyle COOH}{\overset{|}{\underset{|}{CO—HN.CH.CH_3}}}}} + 2NH_3 = \underset{\substack{\text{Glycyl-alanine.}}}{\overset{\displaystyle CH_2.NH_2}{\underset{\displaystyle COOH}{\overset{|}{\underset{|}{CO—HN.CH.CH_3}}}}} + NH_4Cl
$$

$$
\underset{\substack{}}{\overset{\displaystyle CH_3}{\underset{\displaystyle COOH}{\overset{|}{\underset{|}{\overset{\displaystyle CH.Cl}{\underset{|}{CO—HN.CH.CH_3}}}}}}} + 2NH_3 = \underset{\substack{\text{Alanyl-alanine.}}}{\overset{\displaystyle CH_3}{\underset{\displaystyle COOH}{\overset{|}{\underset{|}{\overset{\displaystyle CH.NH_2}{\underset{|}{CO—HN.CH.CH_3}}}}}}} + NH_4Cl
$$
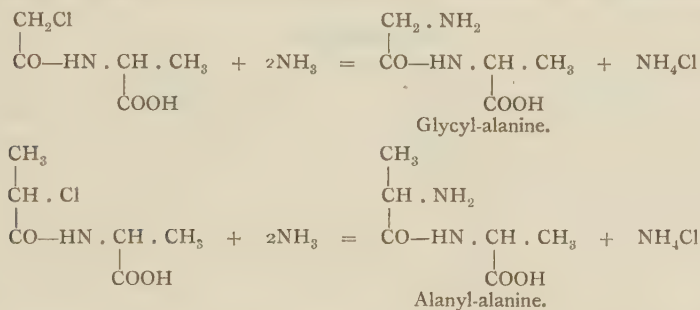
This method is similar to that of the preparation of amino acids. It can be indefinitely extended. The reaction can be repeated with the dipeptide so produced, and then with the tripeptide. The method has been most useful for preparing numerous dipeptides and polypeptides, but is limited in its application partly because the corresponding halogen derivative is not always obtainable and partly because the combination can only be effected at one end of the molecule.

(3) By the action of the acid chloride derivative of one amino acid upon another amino acid, e.g. alanyl-leucine is formed by the action of alanyl-chloride upon leucine :—

$$CH_3.CH(NH_2).CO.Cl + H_2N.CH(C_4H_9).COOH =$$
Alanyl chloride.   Leucine.
$$HCl + CH_3 : CH(NH_2).CO—NH.CH(C_4H_9).COOH.$$
Alanyl-leucine.
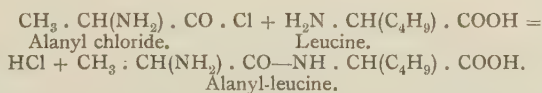
By the action of another amino acid chloride upon this compound a tripeptide will be formed and the continuation of the process will lead eventually to the most complex polypeptides. This is theoretically the simplest and ideal method of preparing polypeptides. In practice,

however, it is difficult to make the acid chloride of amino acids, and on this account the method is not used so much as would be desired.

By the last method the combination of amino acids can be effected in any order, e.g. :—

$$a—b—c—d \quad \text{or} \quad b—c—a—d \quad \text{or} \quad d—b—c—a.$$
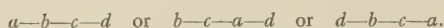
Numerous isomers are thus possible. The actual synthesis of a natural protein will conceivably only be made by chance. At present there is no definite clue of the order of the combination of amino acids in a protein.

The polypeptide constitution of the proteins is confirmed by (1) the hydrolysis of these polypeptides by trypsin and other proteoclastic enzymes into their constituent units in the same way as the natural proteins are hydrolysed, (2) the isolation of polypeptides from the natural proteins, e.g. glycyl-tyrosine.

Glutathione.

The polypeptide, glutathione, isolated by Hopkins in 1921 from yeast, muscle, and other tissues is composed of glutamic acid and cysteine, or cystine. Its constitution[1] has been shown to be glutamyl-cysteine, or diglutamyl-cystine :—

$$
\begin{array}{lll}
\text{CO—NH . CH . CH}_2 \text{ . SH} & \text{CO—NH . CH . CH}_2 \text{ . S—S . CH}_2 \text{ . CH . NH—CO} \\
\text{CH}_2 \quad\quad \text{COOH} & \text{CH}_2 \quad\quad \text{COOH} \quad\quad\quad\quad \text{COOH} \quad \text{CH}_2 \\
\text{CH}_2 & \text{CH}_2 \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \text{CH}_2 \\
\text{CH . NH}_2 & \text{CH . NH}_2 \quad\quad\quad\quad\quad\quad\quad\quad\quad \text{CH . NH}_2 \\
\text{COOH} & \text{COOH} \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \text{COOH.} \\
\quad\text{Glutamyl-cysteine.} & \quad\quad\quad\quad\text{Diglutamyl-cystine.}
\end{array}
$$
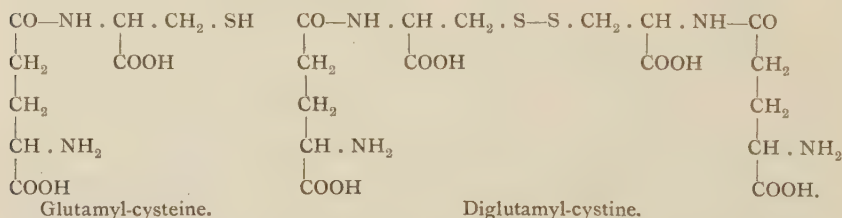
This polypeptide is intimately concerned in the respiration of tissues. Glutamyl-cysteine takes up oxygen and is an oxygen acceptor and changes into diglutamyl-cystine by dehydrogenation. This change is more simply represented as—

$$\text{G . SH + HS . G + O = G . S—S . G + H}_2\text{O.}$$
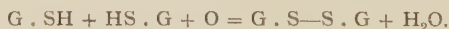
Conversely, diglutamyl-cystine in the absence of oxygen is reduced to glutamyl-cysteine—

$$\text{G . S—S . G + H}_2\text{O = 2G . SH + O.}$$

By this reversible reaction the polypeptide can take up oxygen from the blood stream and pass it to some other compound for oxidation.

Insulin, the active constituent concerned in the metabolism of sugar, absent from the pancreas in diabetes, appears to be a complex polypeptide.

[1] Stewart and Turnicliffe, Biochem. J., 1925, 19, 207.

THE GENERAL REACTIONS OF PROTEINS.

Though proteins are classified into so many groups, they give a series of reactions which are very characteristic and which serve for their identification.

The exact group to which a protein belongs is more difficult to determine, but it can be ascertained by reference to the physical pro-perties of the members of the different groups.

The general reactions depend upon :—

(1) The constituent amino acids (the colour reactions).

(2) The basic character of the units, especially the diamino acid units (the precipitation by alkaloidal reagents).

(3) Their colloidal nature and high molecular weight (coagulation reactions).

All proteins do not give all the reactions ; if a particular amino acid is missing, the colour reaction for that unit will be absent ; some of the coagulation reactions are negative with some of the groups.   Con-sequently it is necessary to perform several of the reactions before the presence of protein is verified.

* The general reactions of a protein are given by a solution of egg-white, which is very conveniently prepared as follows :—

Egg-white is beaten to break up the membranes, filtered through calico, and diluted with nineteen times its volume of water.   A pre-cipitate of ovomucin (formerly regarded as globulin) separates out, but it passes into solution on adding a little salt solution ($NaCl$, Am_2SO_4).

Undiluted egg-white has a faintly alkaline reaction and contains about 10 or 12 per cent. of protein.   The solution may show very faint alkalinity to litmus and contains 0·5 to 1 per cent. of protein.

A. COLOUR REACTIONS.

(1) Biuret.

* To some of the protein solution is added caustic soda and then, drop by drop, dilute copper sulphate solution (1 per cent.), mixing after each addition : a violet colour appears.   Excess of copper sulphate must be avoided, as its blue colour masks the reaction.

This reaction is given by biuret, oxamide, and other compounds. The reaction is due to the presence of two CO—NH groups in the molecule, thus :—

$$\begin{array}{cccc}
\text{CO.NH}_2 & \text{CO.NH}_2 & \text{CO.NH}_2 & \text{HN=C.OH} \\
| & | & | & | \\
\text{CO.NH}_2 & \text{CH}_2 & \text{NH} \quad \text{or} & \text{NH} \\
& | & | & | \\
& \text{CO.NH}_2 & \text{CO.NH}_2 & \text{HN=C.OH} \\
\text{Oxamide.} & \text{Malonamide.} & \text{Biuret.}
\end{array}$$

$$\begin{array}{l}
\text{CH}_2.\text{NH}_2 \\
| \\
\text{CO—NH.CH}_2.\text{CO—NH.CH}_2.\text{COOH} \\
\qquad\text{Diglycyl glycine.}
\end{array}$$
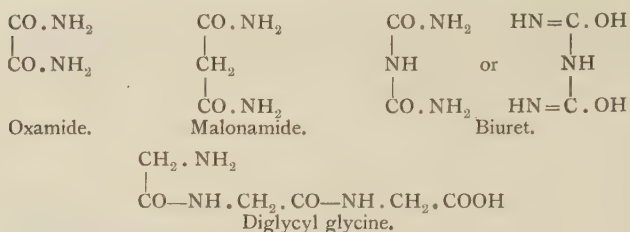
(2) Xanthoproteic.

On heating a portion of the protein solution with concentrated nitric acid, a yellow colour is formed; the colour changes to orange on adding ammonia, or soda, in excess to the cooled solution.

This reaction is most probably due to the formation of nitro compounds with the aromatic units contained in the protein, namely, tyrosine, phenylalanine.

(3) Millon's.

On adding Millon's reagent to some of the protein solution, a white precipitate is formed; it becomes red on heating.

This reaction is due to the presence of tyrosine in the protein. (Compare hydroxy derivatives of benzene.)

(4) Sulphur.

A drop of lead acetate solution is added to some of the protein solution and sufficient caustic soda to redissolve the precipitate which is first formed. A brown coloration, sometimes black, occurs on boiling.

This reaction is due to the separation of hydrogen sulphide from the cystine unit, which gives lead sulphide with the lead acetate.

(5) Adamkiewicz', or Glyoxylic Acid.

If excess of glacial acetic acid be added to the solution and concentrated sulphuric acid be run underneath it, on standing, or on gently shaking, a reddish-violet colour appears at the junction of the fluids which gradually spreads throughout the solution.

It has been shown by Hopkins and Cole that this reaction is due to the presence of glyoxylic acid in the glacial acetic acid; it is therefore better to use a solution of glyoxylic acid instead of glacial acetic which (if kept in the dark) may not contain this substance.

A little glyoxylic acid solution is added to some of the protein solution and concentrated sulphuric acid is run in as before to the bottom of the test tube. The reddish-violet ring as above described slowly forms.

This reaction is due to the presence of tryptophan in the protein molecule. Some substance is formed from the glyoxylic acid which reacts with the tryptophan.

A similar colour is produced on adding commercial sulphuric acid to a protein solution containing a minimal quantity of formaldehyde. This reaction is brought about by the presence of oxidising agents in the sulphuric acid which act upon the formaldehyde (Rosenheim). It is not due to the formation of glyoxylic acid by aldol condensation of formaldehyde and oxidation (Dakin).

It should be noted that this reaction has been used for many years for detecting formaldehyde added to milk as a preservative.

Proteins precipitated by alcohol and washed with ether, give a blue colour when heated with concentrated hydrochloric acid (Liebermann's reaction). A reddish-violet colour, which ultimately becomes brown, is produced on heating proteins with concentrated hydrochloric acid.

These reactions, according to Cole, are due to the presence of tryptophan ; in the first, glyoxylic acid is derived from the alcohol and ether : in the second, furfural is formed from carbohydrate in the protein and reacts with the tryptophan.

The green to blue colour produced when proteins are heated with benzaldehyde, a drop of ferric chloride and concentrated hydrochloric acid— (Reichl's reaction)—is also due to the presence of tryptophan.

(6) Molisch's.

A few drops of a-naphthol solution are added to the protein solution and mixed thoroughly. Concentrated sulphuric acid is run under the solution. At the junction of the two liquids a purple-red ring is formed.

This reaction is due to the formation of furfural from the carbohydrate radicle in the protein and to its combination with a-naphthol.

B. COAGULATION REACTIONS.

(1) Heat.

On heating some of the solution an opalescence occurs, with perhaps a slight precipitate on the surface of the glass. On faintly acidifying it, or another portion, with 1-2 drops of dilute acetic acid and again heating, a cloudiness and then a flocculent precipitate of coagulated protein is formed. This precipitate is not soluble in dilute acids and alkalies in the cold, but it gradually dissolves on heating with caustic soda.

Coagulation did not occur at first as the reaction was alkaline ; it only occurs when the solution is faintly acid.

(2) Alcohol.

A precipitate is formed, if excess of alcohol be added to some of the solution. This precipitate is at first capable of re-solution in water, but on prolonged contact with alcohol it is rendered insoluble, the protein being coagulated.

(2a) Ether. On adding about half a volume of ether to some of the solution and mixing thoroughly by inverting the liquids, a gelatinous solution results, which contains coagulated protein.

(3) Strong Mineral Acids.

Heller's Test.—Concentrated nitric acid is added to some of the solution by means of a pipette, or by gently pouring down the sides of the tube, so that the acid forms a distinct layer below the solution. At the junction of the two liquids a white ring of coagulated protein is formed. The precipitate does not dissolve in excess of the acid, if the liquids be mixed by shaking.

C. PRECIPITATION REACTIONS.

(1) Solutions of Heavy Metals.

Mercuric Chloride.—If 2 or 3 drops of mercuric chloride solution be added to some of the protein solution, a heavy white precipitate of the mercury compound is formed. This dissolves on adding some saturated sodium chloride solution. The mercury compound is reprecipitated from its solution in sodium chloride on adding a few drops of dilute hydrochloric acid.

Copper sulphate, added drop by drop, forms a bluish-violet precipitate which dissolves in caustic soda, giving a violet solution (biuret reaction).

Ferric chloride gives a precipitate, soluble in excess.

Lead acetate and *basic lead acetate* give white precipitates.

(2) Alkaloidal Reagents in Acid Solution.

(*a*) *Hydroferrocyanic acid.* A few drops of glacial acetic acid are added to a little of the protein solution and then, drop by drop, potassium ferrocyanide solution. A voluminous precipitate is formed. This precipitation is less complete in the presence of neutral salts and does not occur in neutral solutions.

(*b*) *Sulphosalicylic acid.* A precipitate is formed on adding a solution of sulphosalicylic acid.

(*c*) *Picric acid.* A yellowish precipitate is formed on adding picric acid to egg-white solution.

(*d*) *Potassio-mercuric iodide (Brücke's reagent).* A whitish precipitate is formed when the protein solution is acidified with dilute hydrochloric acid and a few drops of potassio-mercuric iodide are added.

(*e*) *Trichloracetic acid.* A white precipitate is formed on adding an equal volume of 10 per cent. trichloracetic acid.

(*f*) *Tannic acid.* A brownish precipitate is formed.

(*g*) *Bromine water* gives a white precipitate.

(*h*) *Phosphotungstic acid.* A white precipitate is produced when

phosphotungstic acid is added to the protein solution previously acidified with hydrochloric, or sulphuric, acid.

These reagents are the most commonly employed for removing proteins from solution, e.g. in the analysis of blood.

The tannic acid compound is of commercial importance. Leather is made by tanning skins.

The hydroferrocyanic acid and the sulpho-salicylic acid reactions are often used clinically for detecting protein ("albumin") in the urine.

ESTIMATION OF PROTEIN.

Picric acid is used in the estimation of protein by Esbach's method.

* Some of the solution is poured into the Esbach tube (Fig. 47) up to the mark U and then Esbach's reagent up to the mark R. The tube is corked, the contents are mixed by inverting two or three times without shaking and allowed to

FIG. 47.

stand for 24 hours. The tube is graduated in amounts of protein in grams per litre ; the height of the deposit gives the amount. This method is often employed in estimating "albumin" in urine.

Tannic acid could also be used for this purpose with a similar tube graduated for this reagent.
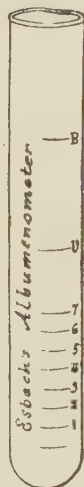
CHAPTER XLI.

ALL proteins are hydrolysed into their constituent amino acids by boiling with concentrated mineral acids, or alkalies, for 6-10 hours. This hydrolysis of the complex protein takes place in several stages, products intermediate between the large protein molecule and the amino acid molecule being formed. These products are metaprotein, proteoses, peptones, and polypeptides. In the formation of metaprotein only a comparatively small change in the protein molecule occurs. Proteoses and peptones are formed by the breaking up of the large molecule into several large complexes, each of which is gradually hydrolysed into smaller complexes, from which the amino acids are finally formed.

The metaproteins, the proteoses, and the peptones still possess some of the properties of a typical protein, but the amino acids do not. The simple polypeptides are intermediate between the peptones and amino acids ; the more complex resemble the peptones, the simpler the amino acids. In all probability peptone is a mixture of polypeptides.

METAPROTEINS.

The most typical metaproteins are formed from the mixture of albumin and globulin of egg-white, or of blood serum, but they are also formed from the other proteins.

Preparation.

Metaprotein is most easily prepared by the action of acid, or alkali, upon proteins. It is formed fairly rapidly at 60° and higher temperatures, more slowly at 37°.

(*a*) *By Acid.*

* (i) Egg-white, or serum, is mixed with ten times its volume of 0·4 per cent. hydrochloric acid and kept in an incubator at 37° for at least 24 hours.

* (ii) Egg-white, or serum, is mixed with one-third of its volume of glacial acetic acid and allowed to stand. The mixture sets to an

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opaque jelly. The opacity is due to the coagulation of the protein by the strong acid. On diluting with water, the jelly dissolves leaving coagulated protein.

(*b*) *By Alkali.*

(i) Egg-white, or serum, is mixed with ten times its volume of 0·1 per cent. sodium hydroxide and kept at 37° for about 18 hours.

(ii) Egg-white, or serum, is mixed with about one-third of its volume of 2N sodium hydroxide. On standing, it sets to a transparent jelly (Lieberkühn's jelly). The jelly dissolves on diluting with water.

Small quantities may be rapidly prepared by adding about one quarter of the volume of dilute acid, or alkali, to 10 or 20 c.c. of an egg-white solution and keeping in a water-bath at 40-50° for 10-15 minutes.

Properties.

The solutions prepared above are known as acid metaprotein, or acid albumin, and alkali metaprotein, or alkali albumin, depending upon whether acid, or alkali, is the solvent.

(1) No coagulation occurs on boiling a portion of the solution.

(2) Metaprotein is insoluble in water.

On carefully neutralising the solutions with 2N acid, or alkali, respectively, the metaprotein is precipitated when the solution is just acid to litmus; it redissolves in an excess of either acid, or alkali. The precipitate is allowed to settle, the bulk of the water decanted and the remainder filtered. The precipitate is washed with water and examined as follows :—

(3) Metaprotein is soluble in dilute acid, or alkali.

A portion of the precipitate will dissolve in dilute acid, or alkali, and will be precipitated on neutralising.

(4) Metaprotein is coagulated by heating in neutral solution.

A portion of the precipitate is suspended in water and heated. Coagulation occurs. This is verified by adding a drop of dilute acid to the cold solution when the precipitate is no longer found to be soluble.

(5) Behaviour towards salt solutions.

Acid metaprotein solutions are precipitated completely on saturating the solution with (*a*) sodium chloride, (*b*) magnesium sulphate, or (*c*) by half saturation with ammonium sulphate. Alkali metaprotein solutions are not precipitated by saturation with sodium chloride, but are precipitated by saturation with magnesium sulphate, or half-saturation with ammonium sulphate.

(6) Both solutions give the colour reactions and some of the other general protein reactions.

PROTEOSES AND PEPTONES.

A mixture of these substances is formed by the hydrolysis of proteins. They are termed albumose, globulose, caseose, etc., fibrin-peptone, gelatin-peptone, histo-peptone, etc., according to the name of the protein from which they arise.

Preparation.

The mixture of proteoses and peptones is most easily prepared by digesting a protein (egg-white, meat, etc.) with 20 parts of ·4 per cent. hydrochloric acid and with about ·01 gm. of pepsin at 37° for several days.

Witte's peptone is a commercial peptone prepared in this way from fibrin. Similar commercial peptone preparations are made from other proteins.

Properties.

The commercial mixtures consist of amorphous powders, white, or pale yellow, in colour, easily and generally completely soluble in water, but sometimes a small residue remains undissolved. A solution of the mixture (5 per cent.) shows the following reactions :—

A. **Colour Reactions.**

* The colour reactions for proteins are generally positive.

B. **Coagulation Reactions**.

* (1) No coagulum is formed on boiling the solution after acidifying with a drop of acetic acid.

* (2) A white precipitate is formed on adding nitric acid, drop by drop, especially in the presence of salts. This precipitate dissolves on heating, but reappears on cooling.

* (3) Alcohol precipitates the proteoses more or less entirely; the precipitate is not coagulated by standing in contact with alcohol and redissolves on adding water.

C. **Precipitation Reactions.**

(*a*) **Heavy Metals**.

* The solution is precipitated by solutions of the heavy metals: copper sulphate, lead acetate, mercuric chloride.

(*b*) **Alkaloidal Reagents**.

* (1) A white precipitate is formed on adding a drop of glacial acetic acid and 2 or 3 drops of potassium ferrocyanide. This precipitate dissolves on heating, but reappears on cooling.

* (2) Tannic acid gives a white precipitate.

* (3) Other alkaloidal reagents also produce precipitates.

SEPARATION OF PROTEOSES AND PEPTONE.

The mixture of derivatives obtained by the hydrolysis of a protein is separated by salting out from the solution. Saturation with sodium chloride, or magnesium sulphate, produces the same effect as half saturation with ammonium sulphate, or zinc sulphate. Ammonium sulphate is used almost exclusively.

(1) Primary Proteoses.

A white precipitate of primary proteoses is formed when an exactly equal volume of saturated ammonium sulphate solution is added to a solution of Witte's peptone (20 or 25 c.c. should be used in testing). If the mixture be well stirred with a glass rod covered at the end with a piece of rubber tubing, the precipitate may gather upon the end of the rod and can be almost completely removed in this way, otherwise it is separated by filtering.

The mass thus collected may be dissolved in warm water. The cold solution will give the previous reactions for the mixture of proteoses and peptone.

This precipitate, according to Haslam, contains three substances :—

α-protoproteose.
β-protoproteose.
Heteroproteose.

The α- and β-protoproteoses are very similar to one another and are easily soluble in water. Heteroproteose is very little soluble in water and can be separated from the others by dialysis ; it is then precipitated from solution.

Heteroproteose and the protoproteoses are more easily separated by means of alcohol. Heteroproteose is precipitated by 32 per cent. of alcohol ; protoproteose is soluble in alcohol up to 80 per cent.

If an equal volume of alcohol be added to the above solution of primary proteoses, the heteroproteose will be precipitated.

(2) Secondary, or Deutero-proteoses.

These proteoses are precipitated by complete saturation of the solution of proteoses and peptone with ammonium sulphate.

The filtrate remaining after the precipitation of the primary proteoses is acidified with a drop of dilute sulphuric acid and saturated with finely powdered ammonium sulphate.[1] A flocculent precipitate comes down and is filtered off.

If it be dissolved in water, it will be found not to give all the above reactions for proteoses, e.g. :—

The reactions with acetic acid and potassium ferrocyanide, concentrated nitric acid, copper sulphate are negative.

[1] 4 gm. to every 10 c.c. of half-saturated solution.

This precipitate also consists of a mixture of at least two deuteroproteoses, α and β, and a third has been described.   They differ in their behaviour towards ammonium sulphate.

Both the primary and secondary proteoses are very indefinite substances and methods have still to be devised for a perfect separation.

PEPTONE.

Peptone is not precipitated by saturation with ammonium sulphate. It therefore remains in solution after the proteoses have been removed.

Its chief characteristic is the biuret reaction :—

A portion of the filtrate is treated with excess of strong caustic soda solution (40 per cent., or solid substance) and a drop or two of 1 per cent. copper sulphate solution.   A pink colour appears, which is characteristic of peptone.   It is necessary to add a large excess of caustic soda, if ammonium sulphate be present in the solution in order to decompose it and in order that the alkalinity should be due to sodium hydroxide ; the alkalinity of ammonia does not produce the colour.

Of the other colour reactions they are sometimes positive, sometimes negative, depending on the peptone.

Peptone is precipitated by some of the precipitating reagents, e.g. tannic acid, phosphotungstic acid, lead acetate, but not by others.

Peptone again is a mixture which has not been perfectly separated ; at least two peptones are present.

CHAPTER XLII.

THE proteins, also the fats and soaps and the polysaccharides, the principal substances with which physiological chemistry has to deal, are colloids. Their properties depend so much upon this fact that it is necessary to examine the nature of colloids and colloidal solutions.

Crystalloids and Colloids.

Thomas Graham between 1861 and 1864, whilst studying the diffusion of dissolved substances through organic membranes, such as parchment paper, found that some substances dialysed, or passed freely through the membrane, but that other substances did not pass through, or passed through very slowly. The substances belonging to the first class were salt, sugar, urea, etc., which crystallised well : the substances belonging to the second class like glue, gelatin, albumin, gum, starch, did not crystallise. He distinguished the two classes as crystalloids and colloids ($\kappa o\lambda\lambda a$ = glue).

Natural and Artificial Colloids.

The substances belonging to the group of colloids show amongst themselves many differences :—

Hot solutions of gelatin, or agar, on cooling form jellies which re-dissolve on warming. Solutions of albumin on heating coagulate, i.e. form an insoluble precipitate. Solutions of gum neither set to a jelly nor coagulate, but always form more or less viscous solutions.

Graham found that substances like silicic acid, ferric hydroxide, etc., substances which are usually insoluble, could be made to form true solutions in their appearance to the eye, and that the solid matter in apparent solution did not diffuse through parchment membranes.

These artificial solutions had one peculiar property : they under-went a marked and irreversible change on the addition of a small quantity of an electrolyte. The solid matter was either precipitated, or

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[1] An excellent description is given by Hatschek, " An Introduction to the Physics and Chemistry of Colloids," from which book most of these notes have been compiled.

the solution set to a jelly; neither the precipitate nor the jelly could be redissolved to form a solution.

Variety of Solvent.

It is now known that other solvents besides water can dissolve substances forming colloidal solutions.

Cellulose dissolved in Schweitzer's reagent, or in zinc chloride, forms a colloidal solution from which the substance is precipitated as a gelatinous mass. Nitrocellulose dissolved in acetic acid, acetone, or alcohol-ether, forms a colloidal solution. Sodium chloride can be made to form a colloidal solution in petroleum ether and the alkali metals in organic solvents.

Sols and Gels.

Graham called the apparent solutions of colloids colloidal solutions, or sols, and the precipitated, or gelatinous substance, gels. We can further distinguish the solvent by prefixing its name, e.g. hydrosol, alcoholgel, etc.

PREPARATION OF ARTIFICIAL COLLOIDAL SOLUTIONS.[1]

A. Colloidal Solutions of Metallic Sulphides.

(a) *Cadmium Sulphide.*—A fine suspension of cadmium sulphate, previously washed with distilled water, is treated with hydrogen sulphide. The solution gradually becomes milky and finally has a yellow colour with a reddish surface. The excess of hydrogen sulphide is removed by a current of nitrogen, or by boiling.

* (b) *Arsenious Sulphide.*—About 1 gm. of arsenious acid is boiled for a few minutes with about 75 c.c. of distilled water; the solution is filtered and allowed to cool. On passing hydrogen sulphide through the cold solution, it turns a yellow-orange colour with a greenish surface.

B. Colloidal Solution of Ferric Hydroxide.

* 1 c.c. of a filtered 33 per cent. solution of ferric chloride is added to 100 c.c. of boiling distilled water. A reddish-brown solution is obtained.

A colloidal solution of ferric hydroxide may also be obtained by dialysing a solution of ferric chloride.

C. Colloidal Solutions of Gold and Silver by Reduction.

1 c.c. of 1 per cent. gold chloride solution is diluted with 25 c.c. of distilled water. 2 gm. of tannic acid are dissolved in 100 c.c. of water.

[1] In preparing artificial colloidal solutions the glass vessels must be absolutely clean, preferably new, and washed with nitric or chromic acid. Freshly distilled water should also be used.

On mixing 1 volume of the gold chloride solution with 3 volumes of the tannic acid, a blue solution is formed. On mixing 1 volume of the gold chloride solution with 1 volume of the tannic acid, a red solution is formed.

Similar solutions may be made by treating gold chloride solution with a solution of 1 gm. of hydroquinone, or pyrogallol, dissolved in 500 c.c. of water.

* Ammonia is added drop by drop to 10 c.c. of silver nitrate solution until the precipitate first formed just redissolves. The solution is diluted with 200 c.c. of water. On mixing equal volumes of this solution with the 2 per cent. tannic acid, a brown solution having a greenish colour in reflected light is formed.

D. Colloidal Solutions of Platinum and Silver by Disintegration.

On forming an arc between two pieces of platinum, or silver, wire under distilled water, i.e. by separating the two poles when a suitable current is passed, a colloidal solution of the metal is formed. The larger particles which are formed settle and can be separated by decantation, or filtration.

The metal, e.g. bismuth or chromium, is ground finely in a ball mill and treated for several days alternately with concentrated alkali and acid. On treating with water, a colloidal solution results.

Colloidal copper and silver solutions are obtained, if distilled water be boiled in copper and silver vessels.

Colloidal lead solutions are formed when water, free from oxygen and from which oxygen is excluded, is kept in contact with lead.

* A suspension of fine particles of kaolin is obtained by shaking some kaolin vigorously with water and pouring off from the larger particles which settle rapidly.

E. Colloidal Solution of Lead Chromate Using Viscous Media.

Sols of lead chromate and barium sulphate can be prepared, if the reactions leading to their formation be carried out in a solution containing a colloid such as a solution of caseinogen.

Colloidal solutions of some inorganic salts may be prepared by dissolving them in glycerol and pouring the solution into water (Craw).

* E.g. if some potassium chromate and lead nitrate be dissolved separately in glycerol, the solutions mixed and poured into water, a colloidal solution of lead chromate is formed.

Detection of Colloidal Solutions.

I. *Dialysis.*

The simplest and most convenient way of showing the presence of a colloid in solution is that of dialysis as used by Graham.

As dialyser, Graham employed a piece of parchment paper fastened between two hoops forming a sort of tray which could be immersed in water, or other liquids. As the object is to obtain as large a surface as

possible, the parchment paper is conveniently made in the form of a sausage skin. The colloidal solution is introduced into the sausage skin, the ends may be tied tightly and the skin is immersed in water, or other liquid, or it may be bent into U shape and suspended in a large tall vessel.

Thimbles made of parchment paper are useful for small quantities of solution, and "soufflet" cases may also be used, especially for testing solutions. Fish-bladder is another material frequently employed.

Collodion thimbles, or tubes, prepared by coating surfaces of test tubes, etc., with a solution of collodion in acetic acid, followed by immersion in water and removal of the tough membrane from the glass, form excellent dialysers.

In all cases a current of water is slowly circulated through the beaker, or other vessel, or the dialyser may be put into several changes of distilled water, e.g. :—

*   (a) Some litmus solution is placed with a drop or two of dilute hydrochloric acid in a parchment paper dish which is allowed to float in a beaker of distilled water. The litmus does not diffuse out, but the hydrochloric acid passes into the surrounding water. It may be tested for by silver nitrate in the presence of nitric acid. If the process of dialysis be continued sufficiently long (repeated changes of water), the red colour will disappear and the litmus will become blue.

*   (b) The same experiment is repeated with a mixture of starch solution and glucose; the former being a colloid does not diffuse out, but the latter, a crystalloid, diffuses out and can be tested for in the surrounding water by Fehling's test.

*   (c) Egg-white solution treated in the same way does not diffuse out through a parchment paper membrane. The surrounding water, if tested for protein by the xanthoproteic, Millon's, and the biuret reactions, will show that protein is absent.

The globulin may be precipitated in the paper dish if the egg-white solution be dialysed long enough, as it is insoluble in distilled water; it dissolves on adding a little salt.

II. *Tyndall Phenomenon.*

If a bright beam of light be passed through a colloidal solution contained in a vessel with parallel sides and the solution be viewed from the side it will appear turbid, sometimes with a coloured sheen.

*   III. Colloidal solutions are often opalescent, e.g. starch, glycogen. Some are coloured and show a pseudo-fluorescence: their colour in transmitted light is different to their colour in reflected light.

*   IV. Colloidal solutions, especially those of natural substances, have a great tendency to froth, if shaken.

V. Colloidal solutions cannot generally be filtered through filter paper and behave like suspensions.

E.g. a suspension of kaolin, prepared by shaking up kaolin with water, on filtration passes through, leaving only the large particles.

Similarly, arsenious sulphide sol passes through filter paper.

NATURE OF COLLOIDAL SOLUTIONS.

Faraday, in 1857, who prepared a colloidal gold solution having a red colour by treating gold chloride with an ethereal solution of phosphorus, expressed the opinion that the gold was suspended in the liquid in an extremely fine state of division.

Colloidal solutions have been shown by various methods to consist of suspensions of extremely fine particles.   The colloidal condition is a state, not a form of matter.

Suspensions and Emulsions.   Suspensoids and Emulsoids.

According as the suspension of fine particles may consist either of solid particles, or of liquid particles, two classes are distinguished :—

(*a*) Suspensoid, in which the particles are solid, rigid, and not deformable.

(*b*) Emulsoid, in which the particles are liquid and deformable.

Most of the natural colloidal solutions are emulsoids ; most of the artificial colloidal solutions are suspensoids.   They are sometimes referred to as reversible and irreversible respectively, this terminology referring to their behaviour with electrolytes.

Continuous and Disperse Phases.

It is usual to refer to the particles in suspension as the disperse phase and the medium in which they are suspended as the continuous phase.   The continuous phase may be more concentrated in the form of a jelly, or even a solid ; the disperse phase will then consist of drops of liquid, or dilute solution, in suspension.

Filtration of the Particles.   Ultra-filtration.

Though the minute particles in a sol cannot be filtered off through filter paper, yet they are retained if they be filtered through paper impregnated with either gelatin hardened with formalin, or collodion (Bechhold), or if they be filtered through a clay filter impregnated with gelatin (Martin).   The solution is forced through these filters by pressure and a clear solution, free from particles, results.

Size of the Particles.

(*a*) Knowing the strength of the gelatin, or collodion, filter, from which the size of the pores can be determined, the size of colloidal particles can be estimated. The pores in a 2·5 and 5 per cent. collodion filter are from 21 $\mu\mu$ to 930 $\mu\mu$.[1] Particles which are retained are probably larger than the size of the pores.

(*b*) In the Tyndall phenomenon the particles in the solution which reflect the light must be smaller than the wave-length of light, i.e. from 450 to 760 $\mu\mu$ for the visible spectrum.

The particles may possibly be molecules with a high molecular weight, e.g. albumin, complex dye-stuffs. In the case of metallic and other inorganic sols, the particles probably consist of aggregates of molecules.

The particles have been shown to behave like gases, filling the space in which they are contained and obeying definite laws.

Visibility of the Particles. The Ultramicroscope.

The particles in a colloidal solution are too small to be seen with an ordinary microscope, but in most cases the particles can be seen with the so-called ultramicroscope. With this instrument a strong beam of light is sent horizontally through the solution, which is viewed with a microscope. The particles reflect the light into the microscope and appear as bright specks. Instead of the ultramicroscope arrangement, many colloidal solutions will show particles by reflected light if a cardioid condenser be used with an ordinary microscope.

Brownian Movement of Particles.

The small particles visible in the ultramicroscope, like many larger particles under a microscope, show Brownian movement.

Non-Settling of Particles due to Electric Charge.

The mere smallness of the particles is not sufficient to account for the long time taken for a suspensoid to settle, nor is the fact that the particles are in Brownian movement.

The non-settling of the particles is due mainly to the fact that they are electrically charged and are thus repelled from one another, preventing coalescence to form larger particles, or aggregates.

Almost any substance in contact with water assumes an electric charge; most substances become negatively charged. The charge can be reduced to zero, or even reversed in direction, by the addition of a suitable electrolyte. The particles in a coarse suspension are also electrically charged.

[1] μ = ·001 mm. $\mu\mu$ = ·001 μ = ·000001 mm.

Determination of the Electric Charge of the Particles.

The electric charge on the suspensoid particles may be determined by placing the sol in a U tube; above the sol on each side is put a layer of distilled water. An electric current is passed through the contents of the U tube, the poles being in the water. The particles will travel to the positive, or negative, pole.

This may also be done on a microscope slide furnished at each side with a platinum wire connected with an electric current. A drop of the sol is put on the slide and the particles, when the current is passed, will travel to one side or the other.

PROPERTIES OF COLLOIDAL SOLUTIONS.

A. Suspensions and Suspensoids.

(1) *Concentration.*—These colloidal solutions are generally very dilute and contain only a fraction of 1 per cent. of solid matter in suspension.

(2) *Osmotic Pressure.*—They have a low osmotic pressure. The freezing-point of the continuous phase is lowered very slightly and the boiling-point is raised very slightly.

(3) *Viscosity.*—The viscosity of a suspensoid sol is only slightly higher than that of water and is proportional to the amount of solid matter present.

(4) *Behaviour to Electrolytes :*—

(*a*) The suspended particles are precipitated immediately, or in a short time, by the addition of a small quantity of an electrolyte, e.g. :—

If a few drops of saturated sodium sulphate be added to colloidal ferric hydroxide solution, or of metallic silver, the solid matter is precipitated.

(*b*) Salts containing divalent ions are more effective than salts with monovalent ions; salts with trivalent more than those with divalent.

$$2 \text{ mol. NaCl} = 1 \text{ mol. BaCl}_2 \text{; } 1 \text{ mol. AlCl}_3 = 3 \text{ mol. NaCl.}$$
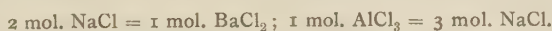
The particles are probably discharged by the oppositely charged ion, so that they no longer repel one another, but coalesce to form larger aggregates.

(5) *Behaviour to other Suspensoids.*

Positively charged suspensoids will precipitate negatively charged suspensoids. Both suspensoids are precipitated together. If the two colloidal solutions contain an equal number of particles with a suitable number of electric charges both are completely precipitated, e.g. :—

* Varying quantities (1, 2, 3 c.c.) of ferric hydroxide sol may be added to varying quantities of arsenious sulphide sol (3, 2, 1 c.c.). Precipitation will occur. The excess of either sol remains and the precipitate contains both substances, as can be seen from the colour of the precipitate and of the solution.

(6) *Influence of Emulsoids.*

* Emulsoids protect suspensoids from precipitation by electrolytes. It seems that a layer of emulsoid particles is formed round the suspensoid and so alters its properties; e.g. if ferric hydroxide sol, or arsenious sulphide, be diluted with (*a*) an equal volume of water, (*b*) an equal volume of albumin solution, and sodium chloride solution be carefully added to each, the amount required to precipitate in (*b*) will be considerably greater than in (*a*).

B. Emulsions.

Emulsions are systems of two liquids insoluble in each other; they consist of comparatively coarse liquid particles of one liquid in another with which it does not mix.

Emulsions are of two kinds: (*a*) a small quantity of a liquid in suspension in a large amount of another liquid; (*b*) a large amount of one liquid suspended in another liquid; in this class the continuous phase must consist of a solution of a colloid such as soap, protein, or saponin.

Formation of Emulsions.

(*a*) An emulsion of oil in water is obtained, if a fine stream of oil be injected into water.

* An emulsion is formed, if an alcoholic solution of oil be poured into water.

* (*b*) Permanent emulsions are formed when colloids are present in a solution and the solution is shaken up with another liquid. The most typical permanent emulsions are observed with fats and oils.

The fats are neutral substances, but generally they contain a little fatty acid, which gives them an acid reaction and causes the formation of an emulsion when they are shaken up with alkali :—

In five test tubes are placed :—

(1)	(2)	(3)	(4)	(5)
10 c.c. H_2O	10 c.c. H_2O	10 c.c. H_2O	10 c.c. H_2O	10 c.c. H_2O
2 c.c. neutral [1] olive oil.	1 drop 8°/$_0$ NaOH	2 drops oleic acid	1 drop 8°/$_0$ NaOH	1 drop 8°/$_0$ NaOH
	2 c.c. neutral olive oil.	2 c.c. neutral olive oil.	2 drops oleic acid 2 c.c. neutral olive oil.	2 c.c. ordinary olive oil.

[1] Neutral olive oil is prepared by dissolving it in ether, shaking up with dilute sodium carbonate solution, washing free from alkali and finally distilling off the ether.

Each is shaken thoroughly. Only in (4) and (5) is a permanent emulsion formed, separation occurring in (1), (2), and (3) after a short time. (5) shows that ordinary fat contains free fatty acid.

The same result can also be seen by dropping a little neutral olive oil and a little ordinary olive oil on to the surface of some dilute sodium carbonate solution in watch glasses. The neutral oil drop remains clear, whilst the ordinary oil drop spreads out and gives a milky emulsion.

The formation of emulsions is due to the fact that a layer of soap, formed by the combination of the free acid with the alkali, is made round the fat particles.

* In the same way an emulsion is obtained when oil, or petroleum, is shaken up with egg-albumin. In both cases a layer of coagulated egg-albumin is formed round each particle.

To prove this :—A little egg-albumin solution is shaken up in a test tube ; a fine layer of mechanically coagulated egg-albumin will be seen to be formed and it rises to the surface on standing.

* Protein solutions have free-surface coatings ; by mechanically shaking, these are heaped up to form solid masses of protein. The following simple experiment demonstrates the surface coating of a protein solution :—

Two beakers are taken ; in the first is placed clean water, in the second egg-white solution On to the surface of each is floated a magnetised needle and a magnet is brought near. In the first beaker, the needle spins round ; in the second, only a slight attraction, or repulsion, is seen. If the beaker be suspended by a wire, in the latter case the whole beaker would swing round, whereas in the former only the needle would rotate. (Ramsden.)

Milk and rubber latex are examples of naturally occurring permanent emulsions. Milk contains fat globules in a solution of the protein caseinogen ; rubber latex contains drops in a solution of vegetable protein.

An extreme case is an emulsion of 99 per cent. of oil and 1 per cent. of soap solution which is of such a consistency that it can be cut into cubes.

Properties of Emulsions.

(a) The properties of the first kind of emulsions in which a small quantity of liquid is present in another liquid—1 part in 10,000—are almost the same as those of suspensoids. The globules show Brownian movement, they are precipitated, or coagulated, by electrolytes and can

be retained by ultra-filters. The particles are comparatively rigid and are separated from one another by thick films, or layers, of the continuous phase.

(*b*) The properties of the second kind of emulsions in which the quantity of disperse phase is large are very different.

(1) *Viscosity.*—They are very viscous, an extreme case being the soap and oil emulsion mentioned above, which is almost a solid.

(2) *Closeness together of the Particles.*—If particles of a solid, or rigid sphere, be put together so that they touch, they will occupy 74 per cent. of the volume. Such a condition gives a thick paste, which is a solid. If particles of a liquid, or a deformable sphere, be put together so that they touch, the particles will become flattened and their face will have the shape of a dodecahedron. The whole system remains a viscous liquid. There is no limit to the ratio of the disperse phase to the total volume.

(3) *Surface Tension of the Continuous Phase.*—In these emulsions the continuous phase must be a solution of an emulsoid colloid. Such solutions froth when they are shaken. Frothing is due to a lowering of the surface tension of the solvent by the substance in solution. This lowering of the surface tension takes place at the points of contact between the two phases, i.e. the interfacial tension is lowered, which prevents tearing of the films of continuous phase between the particles.

(4) *Structure of an Emulsion.*—The globules are flattened and form polyhedra, and they are separated by thin films of continuous phase. The whole system will be represented by a honeycomb structure filled with globules. On shearing, the whole surface of an emulsion becomes enlarged, the polyhedra moving over one another. Surface energy in spite of the lowered surface tension will be developed and it appears as viscosity.

C. Emulsoids.

Silicic acid sol is one of the few examples of an inorganic emulsoid. The organic emulsoids are very various. The types are represented by gelatin and agar, albumin, gum-arabic, cellulose and nitrocellulose solutions.

Preparation of Emulsoid Sols.

(*a*) *Silicic Acid.*—A solution of sodium silicate is treated with excess of hydrochloric acid and dialysed. A clear solution remains in the dialyser.

(*b*) *Gelatin and Agar.*—These substances dissolve in hot water.

(*c*) *Albumin* dissolves in cold water.

(*d*) *Cellulose* dissolves in Schweitzer's reagent, or zinc chloride solution.

(*e*) *Nitrocellulose*[1] dissolves in alcohol-ether, acetone, acetic acid, etc.

General Properties of Emulsoid Sols.

(1) *Concentration.*—These colloidal solutions can be prepared of various strengths and are not necessarily dilute as suspensoid sols.

(2) *.Osmotic Pressure.*—They have a low osmotic pressure.

(3) *Viscosity.*—They have a high viscosity.

(4) *Behaviour to Electrolytes.*—Silicic acid resembles suspensoid sols by being precipitated as a gel with a small quantity of electrolyte.

The organic sols require larger amounts of electrolytes to precipitate them from solution, thus :—

*   (*a*) Sodium chloride is added to soap solution in small quantities at a time and occasionally shaken. The soap is precipitated after a large amount has been added.

*   (*b*) On adding ammonium sulphate to starch solution, precipitation of the starch occurs after a considerable quantity has been added, if the solution be occasionally shaken so as to dissolve the salt.

*   (*c*) If some egg-white solution be saturated with (1) sodium chloride, (2) magnesium sulphate, by grinding it in a mortar with the salt, a small quantity of globulin is precipitated.

The same result is obtained by half-saturating the egg-white solution with ammonium sulphate, i.e. by adding an equal volume of saturated ammonium sulphate solution. On saturating the filtrate with finely powdered ammonium sulphate crystals, the egg albumin is precipitated. This method is employed for separating globulins, which are less soluble, from albumins, which are more soluble and are only precipitated from solution by completely saturating with ammonium sulphate (see under proteins).

(5) *Behaviour towards Suspensoids.*—Suspensoids and emulsoids, if they have opposite electrical charges, mutually precipitate one another. This property is made use of in precipitating proteins from solution (many, if not all, of the alkaloidal reagents act in this way) :—

*   If to some egg-albumin solution, or dilute serum, an equal volume, or more, of colloidal ferric hydroxide be added, and then about ·5 to 1 gm. of sodium sulphate and the mixture be well shaken, a brownish mass containing the protein and excess of ferric hydroxide is precipitated. The filtered solution will not contain protein as shown by the biuret reaction, Millon's reaction, etc.

[1] Cellulose nitrates.

(6) *Electrical Charge.*—The electrical charges on the particles of an emulsoid sol are chiefly due to the reaction of the medium, e.g. albumin in neutral solution is not charged and does not travel in an electric field. Albumin in faintly acid solution has a positive charge and travels to the negative pole. Albumin in faintly alkaline solution has a negative charge and travels to the positive pole.

(7) *Adsorption.*—If an emulsoid sol be precipitated by electrolytes, or by suspensoids, dissolved substances are taken out of solution in the same way as with suspensoids.

Special Properties of Emulsoids.

The properties of emulsoids show many differences among themselves and many differences from the properties of suspensoids and emulsions.

Silicic Acid.

Silicic acid sol on treatment with an electrolyte behaves like a suspensoid ; a small quantity of electrolyte causes gel formation. The gel takes the form of a jelly which gradually becomes more viscous and sets to a hard mass with no separation of water. The change of state is continuous, proceeding until the mass sets.

Thus, if excess of sodium silicate solution of sp. gr. 1·16 be added to 2N hydrochloric acid, an opaque gel containing the salt is formed which gradually becomes more viscous and sets. The rigid gel cannot be redissolved. The colloidal solution thus resembles a suspensoid sol in that the transformation is irreversible.

Gelatin and Agar.

Both gelatin and agar dissolve in hot water. The solution on cooling sets to a jelly. A quite stiff gel is formed by 2 per cent. agar solution. These non-rigid, or elastic, gels can be dissolved again on warming. The transformation is reversible ; they show the phenomenon of hysteresis. The setting-point is influenced by the presence of salts : citrates raise the setting-point, thiocyanates lower it, or may prevent setting.

Albumin.

Solutions of albumin coagulate on heating at temperatures varying from 50-70°. The exact temperature depends upon the amount and kind of salt present ; in the presence of thiocyanates heat, coagulation does not occur even at the boiling-point. The transformation is irreversible.

Solutions of albumin are precipitated by high concentrations of

electrolytes (Na_2SO_4, $(NH_4)_2SO_4$, $MgSO_4$). These precipitates redissolve in water ; the transformation is reversible.

The precipitates formed by $CaCl_2$, $SrCl_2$, $BaCl_2$, become insoluble on standing, whilst the precipitates formed by solutions of heavy metals are insoluble.

Caseinogen, Gum-Arabic.

These sols do not coagulate on heating and do not form gels. The solutions are simply more or less viscous at different temperatures.

Cellulose, Nitrocellulose.

These sols form coherent gels when the solvent is removed by evaporation, or by washing out with water.

Transition of Emulsoids to true Solutions.

Some substances form emulsoid sols in one solvent, but true solutions in another solvent.

E.g. soap in water is an emulsoid sol, in alcohol a true solution ; tannin in glacial acetic acid is a true solution, in water an emulsoid sol.

There are many differences amongst the dye-stuffs ; eosin resembles a true solution ; fuchsin forms an emulsoid sol.

Nature of Emulsoids.

Emulsoids possess many of the properties of emulsions, especially high viscosity, and they show many differences from the suspensoids. Their behaviour can only be explained on the assumption that they are systems of two liquid phases, i.e. as systems consisting of dilute solutions of a colloid containing droplets, or globules, of more concentrated solution.

They differ from emulsions in the ease in which the solvent may pass from one phase into the other. Gelatin sol is a continuous liquid phase containing droplets of higher concentration : gelatin jelly is a continuous solid phase containing droplets of dilute liquid. In dissolving gelatin, sol formation takes place by imbibition of water and swelling ; there is disintegration of the original system.

The effect of salts upon the coagulation of albumin and upon jelly formation is to affect the distribution of the solvent between the two phases. They act by altering the compressibility of water. Solution of an emulsoid generally occurs with contraction.

Properties of Gels.

The gels formed by silicic acid, gelatin, agar, etc., which may contain as much as 90 per cent. of water, possess some of the properties of solids.

They may be put into two groups—the rigid, or nearly non-elastic, gel like silicic acid and the elastic gel like gelatin, collodion, etc.

(1) *Behaviour to Water.*

(*a*) The rigid gel of silicic acid is translucent; on exposure to the air it loses water, becoming opaque, and with loss of more water it again becomes clear. The amount of water present in the solid material corresponds with the tension of aqueous vapour, the ratio of the constituents, silicic acid and water, changing continuously. No definite hydrate is formed, such as occurs with crystals containing water of crystallisation. The formation of many siliceous minerals may be accounted for in this way. The elastic gel, like gelatin, which is reversible, also behaves in a similar manner to water; the amount of water present in it depends on the tension of aqueous vapour.

(*b*) Gelatin behaves differently when immersed in water; it swells and much more water is taken up; it is given off again on exposure to the air. This absorption of water is of great physiological importance.

(*c*) Though an absorption of water and swelling take place when an elastic gel is put into water, the actual volume of the gel and water is less than the total volume of the two substances. There is compression of the water.

The decrease in volume is demonstrated by Hatschek by placing a known weight of gelatin in a pycnometer, filling it with water, and immersing the vessel in water. When the gelatin has swollen, the vessel is taken out of the water, dried, and weighed. There is an increase in weight which shows that water has entered the vessel. To compress water to an extent corresponding to 2 per cent. of the original volume requires 400 atmospheres.

(*d*) Heat is liberated during the swelling of the gels. It has been measured and found to vary from 5-10 gm. calories per gm. of gel.

(*e*) The total volume decreases, but the gel in water swells. This increase in volume has been measured and it has been found that against a pressure of 42 atmospheres a gel will swell by 16 per cent. of its volume; against a pressure of 1 atmosphere the increase in volume is 330 per cent.

From this it can be calculated that 1 gm. of gel on swelling will lift 1 kilo to a height of 3·3 cm.

(2) The elasticity, the optical constants and thermal expansion of gels differentiate them from both liquids and solids.

When not strained they resemble liquids. If stretched, (1) they contract on warming and rapid cooling produces expansion, (2) they become doubly refracting.

They are deformed without change of volume (cross-section ×
length) ; on stretching a cylinder of gelatin its cross-section diminishes
as the length increases.

(3) *Nature of Gels.*—Gels resemble the organic material of plants
in composition ; there is a cell structure. Liquid is enclosed in cells
formed by a solid phase. Gels consist of a solid[1] continuous phase
enclosing a liquid phase.

The reversibility is accompanied by a distribution of water between
the phases and is affected by the presence of salts.

(4) *Diffusion of Substances and Reactions in Gels.*—In dilute gels
diffusion takes place as in water. The rate is slower with strong gels.
The rate of diffusion is affected by various substances : urea, iodides,
chlorides accelerate diffusion ; sodium sulphate, glucose, alcohol, glycerol
retard diffusion.

These substances affect the distribution of water between the two
phases and probably also the relative volumes of the gel wall and the
free liquid. Diffusion takes place chiefly in the liquid. The reaction
does not proceed continuously, but the product if insoluble is deposited
in strata. Many substances can thus be obtained in the form of large
crystals and often spherolites are formed.

Some organic compounds on separation from hot solvents on cooling
first form transient gels which gradually crystallise. Crystalline minerals
may be formed from gels in a similar way.

(5) *Structure of Gels.*—Gels resemble the organic matter of plants
and animals in composition—that is solid matter containing 80-90 per
cent. of water. Cell structure in animals and plants is visible with a
microscope. Though apparent structure can be seen in gels with a
microscope it is not real, but the presence of a structure in gels is
indicated by the diffusion and reaction of substances in gels.

The Phenomenon of Adsorption by Colloids.

The phenomenon of adsorption by colloids is due partly to the large
boundary surface between the particles and partly to the electrical charge
upon the particles. In some cases the first cause may predominate, in
other cases the second cause.

(1) *The Large Boundary Surface.*

The surface of a liquid against its vapour, or another liquid, is in
tension, known as surface tension, or interfacial tension. Such a tension
also exists between a gas and a solid, and a liquid and a solid.

Work is required to produce, or to enlarge, a surface. A surface is a

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[1] In this connection a solid is a substance less deformable than a liquid, but not non-
deformable.

seat of energy and on this account a surface tends to become a minimum. The surface energy is measured by the product of the surface and surface tension per unit length.

The surface tension tends to reduce the surface and establish equilibrium with other forces acting in the body of a liquid.

Gases in contact with a surface produce a lowering of the surface tension, the amount of lowering being characteristic for each gas. With rising gas pressure, or concentration, there is a lowered surface tension. It is accompanied by condensation of the gas on the surface. The same occurs at the boundary of a solid and a gas.

In a froth which has a large surface there is a higher concentration than in the liquid, and with froth formation there is lowered surface tension. There is thus an increase in concentration in the surface, or adsorption with a lowered surface tension. If a dissolved substance in increasing concentration increases surface tension, it is less concentrated in the surface than in the liquid. If a dissolved substance in increasing concentration lowers surface tension, it accumulates on the surface. A small amount of a substance in solution can increase the surface tension only slightly, but a small amount can lower the surface tension greatly.

The amount of adsorption is proportional to the active surface. It proceeds to a definite end point, or equilibrium.

This is expressed mathematically by

$$\frac{y}{m} = ac^{\frac{1}{n}}$$

where m = amount of adsorbent, y = quantity adsorbed, and c is the end, or equilibrium, concentration in the liquid after adsorption. a and n are constants depending on the solution and adsorbent. This equation shows the peculiarities of adsorption.

(2) *The Electric Charge.*

In precipitating colloids by electrolytes, the charge of opposite sign is the effective ion : equi-valent amounts of the ions produce the same effect. In the mutual precipitation of colloids, the precipitation occurs with colloids of opposite charge. In the first case the ion is carried down with the colloid ; in the second case both colloids are precipitated. The large surface of the colloid further influences the electric charge and in the case of emulsoids the viscosity also.

Peculiarities of Adsorption.

(1) *Amount of Adsorption.*

The amount adsorbed from a solution does not increase in direct proportion to the increase in concentration. It thus differs from chemical

reactions, e.g. ten times the concentration produces only four times the adsorption. Relatively more substance is adsorbed from dilute solution than from a concentrated one.

(2) *Different Adsorption from Different Solvents.*

Usually, the adsorption of substances from water is greater than from organic solvents. This peculiarity may be of practical use. Dyes can be removed from aqueous solution completely by charcoal, the particles being concentrated on the surface. On putting the charcoal into alcohol, the dye passes into the alcohol. This is due to the surface concentration of the dye on the charcoal being in excess of that necessary to produce equilibrium between the phases.

(3) *Selective Adsorption.*

Substances are not adsorbed to the same extent; benzoic acid, or salicylic acid, are more adsorbed than acetic acid by charcoal. This selective adsorption has been put to practical use in capillary analysis, e.g. :—

Strips of filter paper are partly suspended in different solutions; the liquid rises into the paper; above a certain height there is only water; the height to which the dissolved substance rises is different; the more adsorbed substance does not rise so high as the less adsorbed.

Lead salts on filtering through paper are retained by the surface of the paper and account for loss in the concentration of the solutions.

(4) *Adsorption by Different Adsorbents.*

Though the adsorbents may differ in active surface, they adsorb the same relative amount of substance: thus if A adsorbs more X than Y, B also adsorbs more X than Y.

(5) *Reactions accompanying Adsorption.*

Chemical reactions may occur at the same time as adsorption, e.g. :—

Alumina adsorbs the acid of congo red at the ordinary temperature without chemical reaction as seen by the colour, which is blue; on warming, chemical reaction takes place; the alumina becomes red in colour, the colour of the salts of congo red.

(6) *Effect of Adsorption on Extraction by Solvents.*

If there is adsorption of one substance by another, repeated extractions must be made to separate them.

(7) *Filtration of Particles through Sand, etc.*

The sand particles having a negative charge will retain a definite quantity of positively charged colloids, such as colloidal ferric hydroxide and some dye-stuffs. This is apparently due only to the discharge of the electric charges on the particles.

CHAPTER XLIII.

FERMENTATION. ENZYMES.

Historical.

THE remarkable change consisting in the formation of alcohol and carbon dioxide from sugar, on account of the effervescence, or apparent ebullition, of the liquid, during the decomposition of the sugar, was called fermentation, from *fervere*, to boil. In the middle ages the decomposition of proteins was recognised as an analogous process to that of fermentation and the terms putrefaction and fermentation were frequently used to denote any process of decomposition. Cavendish showed that carbon dioxide only was evolved during fermentation, but that in putrefaction, hydrogen was also formed. Lavoisier made the first quantitative analyses of the amount of alcohol and carbon dioxide produced from the sugar, but he made no statement how the change was effected.

Thenard, in 1803, noticed that fermenting liquids always gave a deposit and that this deposit resembled brewers' yeast in being an organic substance containing nitrogen. Though the organic nature of yeast was recognised and its microscopical examination was described by Loewenhoek who found it to consist of small round, or ovoid, particles, it was not connected with the process of alcoholic fermentation until 1838. Three observers, Cagniard Latour, Schwann, Kützing, then independently showed that yeast was a living organism, and that alcoholic fermentation was dependent upon living yeast cells.

Neither Berzelius, who attributed the action of yeast to a catalytic force, nor Liebig, would at first admit that fermentation was due to a living force, but gradually they became convinced after the researches of Pasteur, who further showed that the lactic acid fermentation of milk was due to a micro-organism. The souring of wine and the butyric acid fermentation were also found to be due to the action of living micro-organisms.

At about the same time it was discovered that extracts of plants— barley and almonds, and a little later that extracts of animal organs —of the stomach and pancreas, were able to effect the decomposition of the complex compounds, starch, amygdalin, and proteins into simpler ones.

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The effective substance in barley extract was called diastase, that in almonds, emulsin, that in the stomach, pepsin, that in the pancreas, trypsin.

There were thus two varieties of active agents—the one living (yeast and bacteria)—the other not living (diastase, etc.), and they were called respectively organised ferments and soluble, or unorganised, ferments.

Pasteur, who believed in the necessity of life for all the fermentations, was of the opinion that there was something in the yeast, or living cell, which actually produced the fermentation. Traube, in 1858, clearly stated the position that the yeast cell contained a soluble ferment to which the decomposition was due. On account of the confusion of the terms it was suggested by Kühne in 1878 that the soluble ferment should be termed enzyme (from $εν\ ζυμη$, in yeast), which signifies the agent in yeast which causes the fermentation of sugar. The term enzyme has since been adopted as a general term for the soluble, or unorganised, ferments. In France the term " diastases " is used as a general word for enzymes.

Not until 1897 was it definitely shown by Buchner that yeast did contain an enzyme which fermented sugar in the absence of the living cells, and later it was shown that other cells and bacteria also contained soluble ferments. As the term enzyme was used for the active agent in all cases, the term zymase was given to the agent in yeast which produced alcohol and carbon dioxide from sugar.

Preparation of Enzymes.

Enzymes are present in all living cells. They are either excreted in the juices by definite cells, or glands, of the organism, e.g. by the salivary glands, the pancreas, etc. : that is, they act normally outside the cells which produce them (ectoenzymes), or they are not excreted : that is, they act inside the cell envelope (endoenzymes).

For purposes of investigation, in the former case the juices of the glands, such as saliva and pancreatic juice, are collected, or the glands producing the secretion may be extracted with water, or glycerol, to obtain the enzyme. In the latter case, the enzymes are extracted from the cells in which they are present by rupturing the cells so as to obtain their contents.

The cells are ruptured by the following methods :—

(1) By drying. The material is spread out and dried at a low temperature at 20-30° and sometimes subsequently warming the dried mass to 50 or 60°, or the tissue is stirred up with an equal quantity of alcohol, or acetone, and the liquid poured off after a short time of contact.

The dried material is treated with water; the aqueous solution is filtered and precipitated with alcohol.

(2) By autolysis, in the presence of toluene, or other antiseptics. The tissue is minced and suspended in water containing toluene, etc. The enzymes in the cell dissolve the cell membrane and pass into solution.

The autolytic extracts may be mixed with water, or glycerol, and precipitated with alcohol.

(3) By mechanical disintegration; the cells are ground in a mortar with sand. The ground-up mass may be diluted with water, or the liquid contents may be separated from the cell walls by hydraulic pressure.

This method was used by Buchner to show the presence of the enzyme, zymase, in yeast which ferments sugar. The ground-up yeast cells which formed a liquid mass, were mixed with siliceous earth to form a thick paste. The thick paste was pressed in a powerful hydraulic press. The liquid, which oozed out, was filtered (1) through paper and (2) through a clay candle to remove unbroken cells.

The liquids produced by mechanical disintegration may be diluted and precipitated with alcohol.

The alcohol precipitate by either method is dissolved in water, re-precipitated with alcohol, filtered off, washed with alcohol and ether and dried *in vacuo* over sulphuric acid. Too frequent solution and precipitation by alcohol is avoided as much enzyme is lost in the process.

Aqueous, or glycerol, extracts of the dried material, or the fresh gland, also contain the enzymes and may be used directly, as is usually the case when enzymes are to be detected in tissues.

Chemical Nature of Enzymes.

The chemical constitution of enzymes is still quite unknown; they have been supposed to be proteins, nucleoproteins, and carbohydrates from the fact that the enzyme solution gave the reactions of these classes of compounds. The purest preparations of invertase and amylase that have been prepared have contained carbohydrate; the purest preparation of pepsin has not contained nucleoprotein.

Though the chemical nature of enzymes is unknown they belong to the group of colloidal substances : thus, they do not diffuse through parchment paper and other membranes and can be adsorbed on various materials.

Properties of Enzymes.

(1) Enzymes can only be recognised by their activity.

(2) Enzymes are specific in their action. An enzyme acts only

upon one compound, or a group of compounds, such as the fats and pro-
teins. The most striking instance of their specificity is observed in the
a- and β-glucosides. The enzyme maltase acts only upon a-glucosides:
the enzyme emulsin only upon β-glucosides.

(3) Enzymes act by combination, or by adsorption, with the com-
pound upon which they act. From the combination of an enzyme with
the substance upon which it acts and its specific property, arose the
image of Emil Fischer, that the enzyme was to the substance as a key is
to a lock. Only the proper key will open the lock. In illustration of
these properties Armstrong likened the specificity and combination to
the fitting of a glove upon the hand. Only the right-hand glove will
fit the right hand. There may be combination, but unless it is with
every digit there is no enzyme action.

(4) Enzymes act as catalysts, i.e. they increase the rate of a reaction
which is normally proceeding at so slow a rate that it cannot be
detected.

Enzymes, like catalysts, act more rapidly at high temperatures, but
there is a limit to the increase in the rate produced by enzymes.

They are unstable catalysts; they are usually destroyed at a tem-
perature of 56 to 60 or 65°. At 0° their action is nil, or considerably
less than at room temperature; at body temperature and up to 45° their
catalytic action is at the optimum; at higher temperatures it is more
rapid, but the enzyme rapidly undergoes destruction, so that the result
of the action is generally less than at 37° to 45°.

(5) Enzymes are very sensitive to the presence of salts, acids, and
alkalies. Many enzymes, e.g. diastase, will not act unless salt is present.
Some, like pepsin, act only in the presence of very dilute acid ($\cdot 1$ N);
others, like trypsin, act best in the presence of dilute alkali ($\cdot 1$ N). Most
enzymes act best in a very faintly alkaline medium. The action of all
enzymes is stopped by acid, or alkali, exceeding $\cdot 1$ N.

(6) Some enzymes require the presence of particular salts, or other
substances, for their action, i.e. require a *co-enzyme*. E.g. phosphates are
essential in the fermentation of sugar to alcohol and carbon dioxide, the
fat-hydrolysing enzymes require the presence of bile salts, oxidising
enzymes require the presence of iron, or manganese, salts.

(7) Some enzymes in their action are inhibited by other enzymes, or
anti-enzymes.

(8) Many enzymes require liberation from a precursor before they
act—*proenzymes*, e.g. trypsin and its precursor—trypsinogen.

(A full account of the action of enzymes is given in Bayliss'
" Nature of Enzyme Action." Only the general principles of the action
of enzymes can be mentioned here.)

Nomenclature.

Enzymes are designated by the suffix -ase, the first part of the word being that of the name of the substance upon which the enzyme acts. The substance upon which the enzyme acts is known as the substrate, or hydrolyte. Most enzymes act by hydrolysis and are hydrolytic. Those which act upon the carbohydrates are sometimes termed sucroclastic (sugar-splitting); upon fats, lipolytic or lipoclastic; upon proteins, proteolytic or proteoclastic. Other enzymes act by oxidation of the substrate and are termed oxidases. Another group of enzymes acts upon amino groupings forming hydroxy, or keto, groups and enzymes can also remove carbon dioxide from carboxylic acid groups. They may therefore be classified as follows :—

A. *Hydrolytic.*
I. *Sucroclastic.*

ENZYME.	SUBSTRATE.	PRODUCT.
Diastase or Amylase.	Starch, or Amylum.	Dextrin + Maltose.
	Glycogen.	,, ,,
Inulase.	Inulin.	Fructose.
Invertase or Sucrase.	Cane Sugar.	Glucose + Fructose.
	Raffinose.	Fructose + Melibiose { Glucose. Galactose.
Lactase.	Lactose.	Glucose + Galactose.
Maltase or α-Glucase.	Maltose.	Glucose + Glucose.
	α-Glucosides.	α-Glucose.
Emulsin or β-Glucase.	β-Glucosides.	β-Glucose.
	Amygdalin.	Benzaldehyde, HCN, 2 mols. Glucose.
Zymase.	Glucose. Fructose. Mannose. Galactose.	CO_2 + Alcohol.

II. *Lipoclastic.*

Lipase.	Fats.	Glycerol + Fatty Acid.

III. *Proteoclastic.*

Pepsin.	Proteins.	Proteoses + Peptone.
Trypsin.	,,	Amino acids.
Erepsin.	Proteoses.	,, ,,
Papain.	Proteins.	,, ,,

B. *Oxidases.*

Catalase.	Hydrogen Peroxide.	Oxygen.
Peroxidase.	Peroxides :—	
	Hydrogen peroxide or Organic peroxides.	"Active" Oxygen.

ENZYME.	SUBSTRATE.	PRODUCT.
C. *Deaminases.*		
Guanase.	Guanine.	Xanthine.
Adenase.	Adenine.	Hypoxanthine.
{ Amino acid- { Deaminase.	Amino acids.	{ Hydroxy, or Keto- { acids.
D. *Carboxylases.*		
Carboxylase.	Keto acids.	CO_2 + aldehyde.
——	Amino acids.	CO_2 + amines.

This list does not include all the known enzymes; there are many more amongst the carbohydrate splitting enzymes. The lipoclastic enzymes can be subdivided into butyrinase, lecithinase. Xanthine is oxidised to uric acid by uricase and a whole series of enzymes are concerned in the hydrolysis of nucleic acid, each acting upon a particular substrate. They are grouped together as nucleases and would include guanase and adenase as well as nucleotidases, etc.

THE CATALYTIC ACTION OF ENZYMES.

The resemblance of the action of enzymes to that of inorganic catalysts was pointed out by Berzelius. The agent producing the chemical change apparently takes no part in the reaction and can at the end be recovered unchanged. Minute quantities are capable of effecting a large amount of change; as an example may be quoted O'Sullivan and Tompson's statement that invertase can hydrolyse 200,000 times its weight of cane sugar.

The resemblance is most marked if the velocity of the action of enzymes be compared with that of inorganic catalysts, as is shown in the curves in Fig. 48.
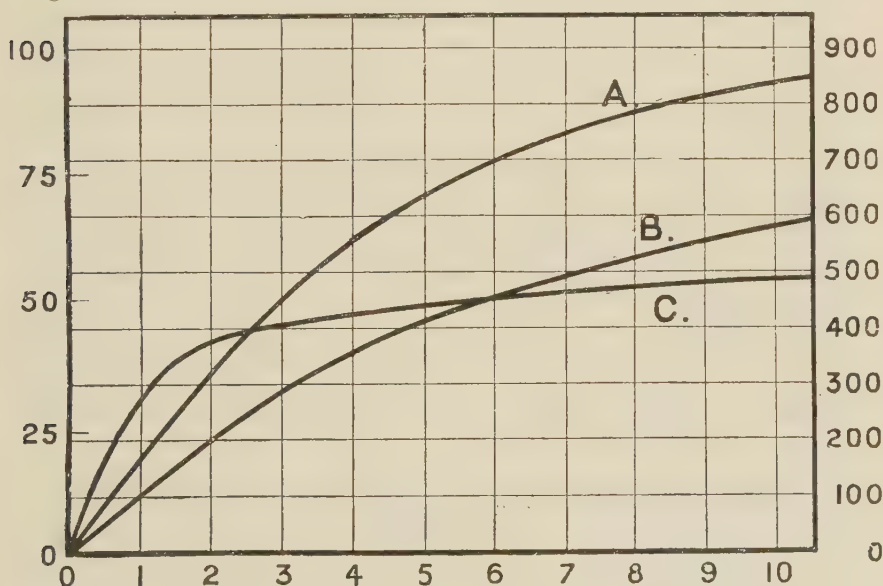
FIG. 48.—From Bayliss' "Nature of Enzyme Action."

Curve B is the velocity of the action of hydrochloric acid upon cane sugar. Curve A is that of invertase upon cane sugar. Curve C is that of trypsin upon caseinogen. This latter curve is the most typical of enzyme action, that of invertase being more exceptional.

The curve B is a logarithmic curve. The enzyme curves deviate from this in two important particulars. They are linear at the commencement and at the end. The cause of the deviation of the enzymic curve from that of a proper catalytic curve has been found to be due to three causes :—

(1) Disappearance of the enzyme during the course of the action. Enzyme solutions, as previously stated, are never pure ; they contain other substances which act upon and remove, or destroy, the enzyme.

(2) Effect of the products of the action ; they hinder the reaction.

(3) Combination of the enzyme with the substrate which also takes an appreciable time.

The linear part of the curve is the result when either substrate is in excess at the beginning, or enzyme is in excess at the end.

THE SYNTHETICAL ACTION OF ENZYMES.

The majority of the chemical changes effected by enzymes are hydrolytic changes. The substrate consists of a compound which can be hydrolysed into two, or more, constituents and in most instances the organic compound has been synthesised from its constituents. The reactions are reversible. The typical example of a reversible reaction is the formation and hydrolysis of methyl acetate :—

$$CH_3OH + CH_3COOH \underset{\rightarrow}{\overset{\leftarrow}{=}} H_2O + CH_3 . COOCH_3.$$
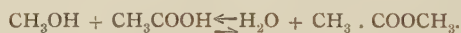
These reactions have been measured and it has been found that an equilibrium is reached from whichever side the reaction is started when the composition of the mixture of the four substances is

$\frac{2}{3}$ mol. ester + $\frac{2}{3}$ mol. water = $\frac{1}{3}$ mol. alcohol + $\frac{1}{3}$ mol. acid.
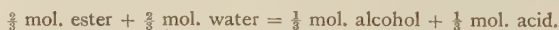
Other similar reactions have also been measured and their equilibrium positions have been determined. Reversible reactions proceed according to the Law of Mass Action. The effect of a catalyst upon reversible reactions, as it effects the reaction to the same degree from both sides, is not to alter the position of equilibrium of the reaction. The final position in the case of enzymes is usually reached when the products of hydrolysis make up over 90 and sometimes nearly 100 per cent. of the mixture. This is on account of the large proportion of water present. Enzymes as catalysts should therefore accelerate the reaction in both directions, i.e. be capable of synthesising the compounds which they hydrolyse. The synthetic power of enzymes has been demonstrated in only a few cases, e.g. that of maltase by Croft Hill, of lipase, of emulsin, and of trypsin. The demonstration of the synthetical action of enzymes is difficult as the equilibrium point is generally so near the point of complete hydrolysis. It can be shown most easily in the cases of lipase and of emulsin.

THE MEASUREMENT OF THE ACTIVITY OF ENZYMES.

In order to determine the activity of an enzyme solution four factors must be taken into account. This was first clearly established

by Kjeldahl in 1879 in the case of the diastatic enzyme of malt, namely :—

(1) The temperature at which the action takes place.
(2) The time during which the enzyme acts.
(3) The amount of enzyme solution.
(4) The concentration of the substrate solution.

The amount of enzyme solution must be small in comparison with the amount of substrate and the change must not exceed 30-40 per cent. of the total change.   These statements correspond to the curve of the catalytic action of enzymes.   The curve is linear at the commencement and again at the end. The measurement is proportional only during the linear part of the curve where the amount of enzyme is small and the substance large in comparison. The linear portion of the curve corresponds to 30-40 per cent. of hydrolysis.

The temperature and the concentration of the substrate are fixed, and by fixing either the time, or the amount of enzyme solution, the fourth factor can be determined.   As the basis of comparison, it is best to determine the time taken to effect an equal change.   This is most important where the reaction takes place in stages ; comparable values can be obtained only in this way.   More frequently, the amount of enzyme required to produce an equal change, or the amount of change produced by equal amounts of enzyme solution in a given time, is determined.

The measurements are made either by chemical methods, or by physical methods, depending upon the properties of the substrate and the products.

DEMONSTRATION OF THE ACTION OF ENZYMES.

Since enzymes can only be recognised by their action, their demonstration necessitates the knowledge of the chemical and physical properties of the compounds upon which they act ; e.g. starch and its products maltose and dextrin, fats and their products glycerol and fatty acids, proteins and their products the proteoses, peptones, and amino acids.   Either the disappearance of substrate, or the appearance of the products, or both, may be demonstrated.

Frequently, the amount of enzyme in a solution, or in a preparation, is very small and a considerable time must be allowed before its action can be demonstrated, e.g. from 1 day to 3 or 4 days.   Under these conditions an antiseptic, preferably 1 per cent. of toluene, or chloroform, is added to prevent the action of bacteria.   The antiseptics destroy the bacteria, or inhibit their growth ; they have no action on the enzyme.   In all cases a control must be carried out at the same time.

The control is best obtained by the use of boiled solution of the enzyme. The enzyme is so destroyed and the solution shows no action.

*

I. **Diastase, or Amylase.**

The enzyme diastase, or amylase, is formed during germination of barley grains to produce malt. Malt is thus a good source of diastase.

Malt Diastase.—Malt is prepared by steeping barley, or other seeds of cereals, in water and allowing them to germinate in a warm place until the plumules have reached a length of about ½ inch. The sprouted grain is dried and cured in a kiln. The composition of the grain alters under these conditions: the amount of starch decreases, the amount of reducing carbohydrates increases. Its colour is light to dark yellow. The seeds should break easily, should have a white interior and a sweet flavour. Malt should be free from broken and damaged seeds and the dried rootlets.

Malt extract is prepared from the dried material by treatment with water; the aqueous solution may be evaporated to dryness. Its chief use is in brewing, but it is used in medicine as a food for its high content in maltose and for its diastatic action; some varieties of malt extract contain no diastase, as they have been boiled.

An active diastase is prepared by treating malt, or ground barley, with 2-4 parts of 20 per cent. alcohol, for 24 hours. The extract is precipitated by adding not more than 2½ volumes of alcohol. The precipitate is rapidly treated with absolute alcohol and ether and dried *in vacuo* (Lintner).

Diastase is secreted by the salivary glands, and by the pancreas.

* A 1 or 2 per cent. filtered extract of malt may be used for the demonstration of diastase.

A 1 or 2 per cent. solution of soluble starch, or starch paste, is used as substrate.

5 c.c. of starch solution are placed in each of two test tubes.

5 c.c. of malt extract solution is added to one; 5 c.c. of boiled malt extract solution is added to the other to act as control. Both tubes are placed in a water-bath at 40°.

The action of diastase is tested for by taking out a drop from each tube at intervals of 1 or 2 minutes and testing the reaction with iodine solution. A series of drops of iodine solution are conveniently placed upon a white plate for the purpose.

Drops from the control tube will always give a blue colour. Drops from the enzyme tube will at first give a blue colour, later a reddish-brown, and finally no colour with the iodine, showing that the starch has disappeared.

After about 5 minutes, the contents of each tube are tested for reducing sugar with Fehling's solution. No reduction is shown by the control tube, marked reduction by the enzyme tube.

*

II. Invertase.

The best source of invertase is yeast from which it may be prepared by several methods.   *Method of Autolysis.*—It is most conveniently prepared by grinding 500 gm. yeast with 30 gm. of calcium carbonate into a thick paste and placing the paste in a wide-mouthed bottle.   25 c.c. of chloroform are added and it is kept for 3-4 days in a warm room.   The solution is filtered from the insoluble matter and treated with an equal volume of alcohol.   The precipitate is washed with alcohol and ether and dried *in vacuo* over sulphuric acid.

A ·1-1 per cent. solution of the preparation is used to demonstrate the action of invertase.   Autolysed yeast, if diluted about 100 times with water, also serves for showing the presence of invertase.

*   A cane-sugar solution of 1 per cent. is prepared as substrate ; 5 c.c. of the invertase solution are added to 5 or 10 c.c. of the cane sugar solution ; at the same time 5 c.c. of boiled invertase solution are added to another 5 c.c. of cane sugar solution to act as a control.

The solutions may be kept for about 5 minutes at room temperature, or at 40°.   They are tested with Fehling's solution.   Reduction only occurs in the first tube, due to the formation of glucose and fructose.

III. Emulsin.

*

Emulsin is prepared most easily from almonds.   The almonds are ground and the oil is pressed out.   The residual cake is treated with water at room temperature.   The filtered solution is acidified with acetic acid (2 drops per 100 c.c.) to precipitate proteins.   The filtrate is treated with an equal volume of alcohol.   The enzyme preparation is thrown down, washed with alcohol and ether and dried.   The precipitate can be immediately redissolved in water and used in experiments with emulsin.

*   A substrate of 2 per cent. salicin is conveniently used.   5 c.c. of salicin solution are placed in each of two test tubes.   To the one are added 5 c.c. of emulsin solution ; to the other are added 5 c.c. of boiled emulsin solution.   The two tubes are placed in a water-bath at 40° for 15·30 minutes, or longer ; in the latter case 1 per cent. of toluene should also be added.   The solutions are tested with Fehling's solution.   Reduction occurs in the first tube in which the salicin has been hydrolysed to saligenin and glucose.

Amygdalin may also be used as substrate and the formation of hydrogen cyanide tested for with picric acid paper.[1]   This method has been used by Armstrong for detecting emulsin in plants.   The plant leaf, etc., is put into a small test tube containing a drop of chloroform, and a few drops of amygdalin solution ; the test tube is closed with a cork, to which is attached a piece of picric acid paper.   If emulsin be present, the paper becomes brick-red in colour from the action of hydrogen cyanide.   In a similar way the presence of amygdalin, of emulsin together with amygdalin, may be tested for.

[1] Filter paper moistened with a solution of 1 gm. picric acid + 10 gm. of Na_2CO_3 in 100 c.c. of water.

IV. **Lactase and Maltase**.

Since both lactose and maltose reduce Fehling's solution, their hydrolysis by enzymes is difficult to demonstrate. It can only be satisfactorily demonstrated by the measurement of the reducing power. Lactose and maltose do not reduce Barfoed's reagent, which is reduced by glucose and monosaccharides. Hence this reagent will serve to show the presence of these enzymes.

V. **Zymase** (Yeast).

Yeast contains a mixture of several enzymes. Its principal enzyme is zymase, which acts upon the four natural hexoses. It contains also maltase and invertase, but it does not contain lactase. Lactase is only present in special yeasts, such as kefir. It is owing to the presence of maltase and invertase that yeast is able to ferment maltose and cane sugar and convert them into alcohol and carbon dioxide. Lactose is not fermented, as it is not hydrolysed into its constituent monosaccharides. Before alcoholic fermentation can occur hydrolysis into monosaccharides must take place.

The action of yeast upon the sugars is most conveniently demonstrated with a series of Einhorn fermentation tubes (p. 292). They are filled with 1 per cent. solutions of glucose, fructose, galactose, maltose, cane sugar and lactose, and a small piece of yeast is added to each. Fermentation proceeds slowly, but in 12 hours it will be observed that all the sugars except lactose have been fermented and that galactose is fermented more slowly, as shown by the smaller volume of carbon dioxide evolved.

The presence of zymase in the yeast can be shown either by preparing yeast juice by Buchner's method (p. 400), or by preparing maceration extract by Lebedeff's method. Fresh yeast is carefully dried. 100 gm. of the dried material are treated with 300 c.c. of water for 2 hours at 37°. The mixture is filtered rapidly on a large folded filter paper and the filtrate is collected in a vessel in ice.

Portions of 5 c.c., or 10 c.c., are added to 1 per cent. solutions of glucose, fructose, and the other sugars in Einhorn fermentation tubes. After some hours the formation of carbon dioxide will become visible.

Alcoholic Fermentation.

The mechanism of alcoholic fermentation is much more complicated than the simple production of alcohol and carbon dioxide from sugar by the action of zymase. It has been shown by Harden and Young that phosphates played an essential rôle in the process and that the zymase could be separated by filtration through a gelatin filter into two parts—

the actual zymase—and a co-ferment in the filtrate. Neither alone would cause alcoholic fermentation, but the two together would actively decompose sugar. The co-ferment appears to be phosphate, which, in the reaction, is esterified to hexose phosphate. The reaction is believed to be :—

$$2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + 2H_2O + C_6H_{10}O_4(PO_4R_2)_2$$
$$C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2PO_4HR_2.$$
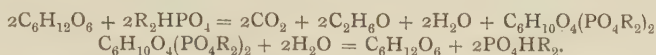
It was shown by Pasteur that other substances in small quantities were formed during alcoholic fermentation, such as glycerol, succinic acid, fusel oil. Of these, only glycerol arises from the sugar. Yeast contains numerous enzymes, invertase, carboxylase, and a reducing enzyme, reductase. Carboxylase acts especially upon ketonic acids, such as pyruvic acid :—

$$CH_3'. CO . COOH = CO_2 + CH_3 . CHO$$
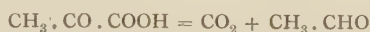
and yeast reductase will convert aldehyde to alcohol :—

$$CH_3 . CHO + H_2 = CH_3 . CH_2OH.$$
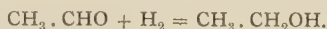
It thus would appear that the alcohol originated from pyruvic acid through acetaldehyde. The formation of aldehyde was demonstrated by Neuberg by adding bisulphite to a fermenting solution. Acetaldehyde bisulphite was formed. At the same time there was a larger production of glycerol. It is not possible to give the exact intermediate stages between sugar, alcohol, and glycerol, but it appears that the sugar molecule is decomposed into 2 molecules of a 3 carbon atom compound. It appears not to be glyceraldehyde. This unknown compound is probably methylglyoxal, which can be reduced to glycerol, or oxidised to pyruvic acid. The stages in alcoholic fermentation are thus probably :—

hexose → 2 × methyl glyoxal → glycerol

pyruvic acid → acetaldehyde + CO_2 → ethyl alcohol.
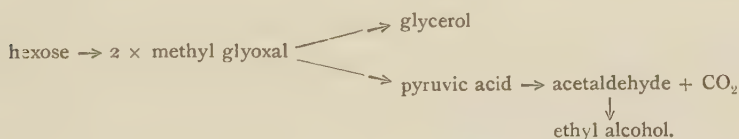
Under ordinary conditions the reaction gives chiefly ethyl alcohol and carbon dioxide. Under the influence of sulphite, the aldehyde is fixed and the reducing enzyme reduces the intermediate substance to glycerol. The reaction producing glycerol has become of technical importance. During the war 1,000,000 kilos. of glycerol were produced in Germany per month.

Fusel oil and succinic acid arise from amino acids in the barley, or

potato, or other source of carbohydrate.   Valine, leucine, and isoleucine and glutamic acid lose carbon dioxide and ammonia, e.g.

$$\begin{array}{l} CH_3 \\ {>}CH.CH_2.CH(NH_2).COOH \\ CH_3 \end{array} = \begin{array}{l} CH_3 \\ {>}CH.CH_2.CH_2OH + NH_3 + CO_2 \\ CH_3 \end{array}$$
$$\text{Leucine.} \qquad\qquad\qquad \text{Iso-amyl alcohol.}$$

$$HOOC.CH_2.CH_2.CH(NH_2).COOH + 2O = HOOC.CH_2.CH_2.COOH + NH_3 + CO_2.$$
$$\text{Glutamic acid.} \qquad\qquad\qquad\qquad \text{Succinic acid.}$$
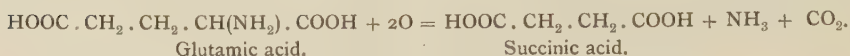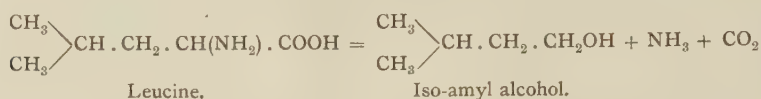
Pyruvic acid is also probably a stage in the production of lactic acid from sugar by fermentation :—

$$CH_3.CO.COOH + H_2 = CH_3.CHOH.COOH.$$
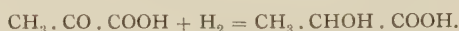
Specificity of the Action of Enzymes.

5 c.c. of diastase solution are added to 5 c.c. of cane sugar solution and kept at 40° for some time.   There is no conversion of cane sugar by diastase into glucose and fructose, as shown by testing for reducing sugar with Fehling's solution.

The same experiment is performed with 5 c.c. of salicin solution instead of cane sugar.   Again, there is no reduction of Fehling's solution.

In the same way the action of 5 c.c. of the invertase solution is tested upon 5 c.c. of starch solution at 40°.   There is no hydrolysis of starch by invertase.   If any action occurs, it is due to impurity in the invertase solution, i.e. to its containing a little diastase.   It is very difficult to obtain enzyme solutions which contain only one enzyme. Most cell contents contain a mixture of enzymes.

5 c.c. of emulsin solution will not hydrolyse 5 c.c. of starch solution, or 5 c.c. of cane sugar solution.

In the experiments with yeast, neither invertase, nor maltase, nor zymase acted upon lactose.

VI. Lipase.

Preparations of lipase are most conveniently obtained from castor-oil seeds and from pigs' pancreas :—

Lipase from castor-oil seeds.
The seeds are shelled, freed from oil by pressure, or by treatment with ether, or petroleum ether, and finely ground up with ·1 N acetic acid.
The lipase is liberated by the treatment with acid.   The insoluble matter is filtered off and washed free from acid.   A suspension of it is made in water.

As substrate for the action of lipase neutral olive oil (see p. 388), an emulsion of egg-yolk in water, milk or esters, such as ethyl butyrate, may be used.   Hydrolysis occurs with the formation of fatty acids which are recognised by the acidity of the solution.

* The presence of lipase in castor-oil seeds may be demonstrated upon the oil in the seed as follows: 1 gm. of seed, freed from shell, is ground up with 25 c.c. of water saturated with chloroform; two equal parts of the suspension (10 c.c.) are placed in two test tubes and to each is added 1 c.c. of dilute acetic acid to liberate the enzyme. One portion is immediately boiled. Both test tubes are kept at 40° for half to one hour. A few drops of phenolphthalein are added and they are titrated with ·1 N alkali. More alkali will be required to neutralise the acid in the tube containing unboiled enzyme showing that fatty acids have been formed.

VII. **Proteoclastic Enzymes**.

Proteoclastic enzymes may act only in neutral, acid, or alkaline solution.

Pieces of fibrin, or pieces of coagulated egg-white, are most conveniently used as substrate.

In order to test for the proteoclastic enzyme under the different conditions, six tests will be required :—

(1) 5 c.c. of enzyme solution + 5 c.c. of water.

(2) 5 c.c. of enzyme solution + 5 c.c. of $0·1 N$ HCl.

(3) 5 c.c. of enzyme solution + 5 c.c. of $0·1 N$ Na_2CO_3.

(4), (5), (6) as above with boiled enzyme solution.

To each tube is added a piece of fibrin. The fibrin dissolves in the presence of enzyme.

Further experiments are given under "Digestion."

VIII. **Oxidases.**

A. *Catalase.*

A catalase is present in most animal and vegetable tissues. Solutions may be prepared by extracting the tissues with water; the extracts are usually not very active and a piece of tissue is used directly.

Since catalase acts upon hydrogen peroxide with the formation of oxygen, only hydrogen peroxide can be used as substrate.

* E.g. a piece of liver is placed in a test tube and covered with a dilute solution of hydrogen peroxide. An evolution of oxygen occurs.

B. *Peroxidase.*

Peroxidases are very abundant in plant tissues. Active solutions are best prepared from horse-radish, potato, or fungi, by grinding up the material, treating with water, and filtering from insoluble matter.

A substrate is usually present in the plant tissue together with the enzyme. On bruising the tissue, it becomes brown like the cut surfaces of apples and pears, or it may become blue, or red, as in some species of fungi. The substrate is a dihydric, or trihydric, phenol—such as hydroquinone, or pyrogallol. In the tissue an organic peroxide, or oxygenase, is also frequently present. On bruising the tissue, oxygen is taken up from the air and the peroxide is formed. The peroxidase acts upon the peroxide giving "active," or nascent, oxygen, which oxidises the substrate. These oxidases are sometimes called direct oxidases.

Sometimes the colour is only given after hydrogen peroxide, or other peroxides (especially organic peroxides), such as are present in oil of turpentine which has been exposed to the air, are added. Such oxidases are called indirect oxidases.

For purposes of demonstration a variety of phenolic substances are used as substrate.

(1) Guaiacum. A freshly prepared 1 per cent. solution in alcohol (tincture of guaiacum). It changes to blue on oxidation.

(2) Guaiaconic acid, the constituent of guaiacum. A ·5-1 per cent. solution in alcohol.

(3) a-naphthol. A 1 per cent. solution in equal parts of water and alcohol. When oxidised, it becomes lavender in colour. This substrate has been largely used in botanical work.

(4) Guaiacol. A 2 per cent. solution in alcohol. It is oxidised to tetraguaiacoquinone, which is red.

(5) Benzidine. A 1 per cent. solution in 50 per cent. alcohol. It becomes blue on oxidation and a brown precipitate is formed.

(6) p-phenylenediamine hydrochloride. A 1 per cent. solution in water. It becomes greenish in colour.

(7) Indophenol. A 1 per cent. a-naphthol solution in 50 per cent. alcohol and a 1 per cent. aqueous solution of p-phenylenediamine hydrochloride are required. 2 or 3 drops of each of these are added to the enzyme solution, which is made faintly alkaline with sodium carbonate. A purple solution results.

* A few drops of any of these reagents are added to about 5 c.c. of the oxidase solution to which a few c.c. of hydrogen peroxide have been added. The colour slowly forms.

* The direct oxidase may be observed in potato: a drop of guaiacum solution is placed upon the cut surface, in a short time it becomes blue.

The presence of an oxidase in minced tissues is readily detected by the indophenol reaction as shown by Vernon.[1] The reaction takes place according to the equation :—

$$C_6H_4(NH_2)_2 + C_{10}H_7OH + O_2 = C_6H_4 \begin{array}{c} NH_2 \\ N=C_{10}H_6O \end{array} + 2H_2O$$

p-phenylene- α-naphthol.
   diamine.
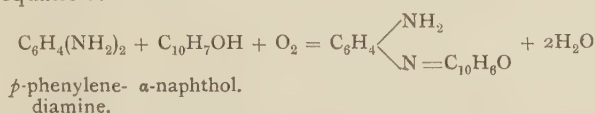
The substrate consists of ·144 per cent. solution of α-naphthol (·oi M) and ·11 per cent. *p*-phenylenediamine (·oi M) in 50 per cent. alcohol. 5 c.c. of the solution together with about 5 c.c. of ·1 per cent. sodium carbonate solution are poured upon ·5-1 gm. of minced tissue in a flat dish (a Petri dish 8·8 cm.) and well stirred with the tissue. The indophenol begins to form almost at once.

[1] J. Physiol., **42**, 402.

CHAPTER XLIV.

I. PROTAMINES.

THE protamines occur in ripe fish sperm in which they are present as salts of nucleic acid. Salmine, the first known member of the group, was discovered by Miescher in salmon sperm. The other members have been isolated from the sperm of other fishes by Kossel and his pupils to whom our knowledge of these proteins is almost entirely due. They are named according to the fish from which they are obtained, e.g. sturine from sturgeon, clupeine from herring, scombrine from mackerel, cyprinine from carp.

They are composed principally of diamino acids, especially arginine, which in some cases makes up over 80 per cent. of the molecule. It seems that they are the diamino acid constituents of muscle protein, since the testicles grow at the expense of the muscles in the spawning season, the fish taking no food and living upon the mono-amino acid portion.

The protamines in the free state have not been much investigated. They are strong bases, blueing litmus, and absorb carbon dioxide from the air. They are easily soluble in water, but insoluble in alcohol and ether. They are not coagulated by heating, do not diffuse, give the biuret reaction, sometimes other colour reactions, and are laevorotatory.

They form salts with acids of which the sulphate is the principal. The chlorides, the carbonates and nitrates are easily soluble. They form insoluble double salts with platinum chloride and mercuric chloride.

They dissolve copper hydroxide giving solutions of a violet colour.

They are precipitated by alkaloidal reagents in neutral, or faintly alkaline, solution and are precipitated from solution by salts. They give precipitates with other proteins, excepting the secondary proteoses, peptones, and polypeptides.

They are precipitated from solution by a solution of sodium nucleate as protamine nucleate.

They contain 25-30 per cent. of nitrogen in their molecule, no sulphur and no phosphorus.

II. HISTONES.

The group of proteins termed histones was established by Kossel, who isolated a histone from the red blood corpuscles of the goose.

Other members of the group have since been isolated from the unripe testicles of fish and from the thymus.   During maturing of the sperm the histone in some cases remains unchanged, but in other cases changes into protamine.

The various histones vary considerably in properties.   They contain about 18-19 per cent. of nitrogen ; some contain sulphur, others do not.

They are basic and are intermediate between albumins and globulins and the protamines, yielding on hydrolysis a larger proportion of arginine than albumins and globulins, but less than the protamines.

Their properties resemble in part the coagulable proteins, in part the protamines, and in part the proteoses.

Neutral solutions, free from salt, are precipitated by ammonia, and also precipitated by caustic alkalies and alkaline earths.   Towards nitric acid they behave like the proteoses.   They are precipitated by the alkaloidal re-agents also in neutral solution.   Like the protamines, they are precipitated by albumin and primary proteoses.   They are not coagulated by heat, but coagulation occurs in the presence of salt.   The coagulum dissolves in dilute hydrochloric acid.

Globin.

Globin, the protein moiety of the conjugated protein, hæmoglobin (p. 423), has been considered to be a histone, though in its properties it has many points of difference.

Globin dissolves in water and differs from histones in that the neutral solution, in the absence of salts, gives a precipitate which is readily soluble in excess of ammonia, and that ammonium chloride only precipitates it when a large excess of ammonia is not present.

Globin gives most of the colour reactions of proteins ; it is not precipitated by most of the heavy metals.

It contains about 17 per cent. of nitrogen of which 29 per cent. is in the form of diamino acids.   In this respect it resembles the histones, but the chief diamino acid is histidine, not arginine.

III. THE COAGULABLE PROTEINS.
ALBUMINS AND GLOBULINS.

These proteins generally occur together in most tissues and fluids of animals, e.g. in egg-white, blood, and muscular tissue.   They are also present in various parts of plants, especially in the fruits and seeds.

The coagulable proteins are the most typical proteins and are often called native proteins.   They have the common property of being changed into insoluble modifications when their solutions are heated to boiling in the presence of a little acetic acid.   The insoluble form is also present in the various animal tissues—such as muscle.

The chief distinction between albumins and globulins is their

behaviour towards water and solutions of salts.   They can be separated from solution in an unchanged condition in this way.   A large number of salts have been used for this purpose, the principal ones being sodium chloride, magnesium sulphate, and ammonium sulphate.

Albumins are soluble in water and in dilute salt solutions. Albumins are not precipitated by saturating their aqueous solutions with sodium chloride, or magnesium sulphate, nor by half-saturation with ammonium sulphate (i.e. adding an equal volume of saturated ammonium sulphate), but they are precipitated by saturation of the solution with ammonium sulphate.

Globulins are insoluble in water, but soluble in dilute salt solutions.   Globulins are precipitated from dilute salt solutions by saturation with sodium chloride or magnesium sulphate, or by half-saturation with ammonium sulphate.[1]

There are, however, several globulins which behave slightly differently by being soluble in water, or by being precipitated with less salt than is required for complete saturation.   These are atypical globulins.

Several albumins have been prepared in a crystalline state, e.g. ovalbumin, serum albumin.   Many of the globulins found in seeds and nuts separate from warm salt solutions in a crystalline condition, e.g. edestin, excelsin.

Coagulable proteins are present in various parts of plants, but are particularly abundant in oil and leguminous seeds, in which they form the reserve protein of the endosperm.

Vegetables and fruits contain from 1-1·5 per cent. of protein, the edible portion of beans about 7 per cent.   Dried peas, lentils, and beans contain up to 25 per cent. protein.   Nuts contain 15-28 per cent.

Globulin forms the greater part of the protein of the seeds, but small amounts of albumin are also present.

The properties of the various coagulable proteins are best studied in connection with the examination of the separate tissues.   (See later.)

IV. GLIADINS AND GLUTELINS.

These proteins are contained in cereals and have peculiar properties.

The gliadins are insoluble in water and strong alcohol, but dissolve in 70-80 per cent. alcohol.

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[1] The amount of salt required to saturate an aqueous solution is
   3·6 gm. of sodium chloride for every 10 c.c.
   10·2 gm. of cryst. magnesium sulphate for every 10 c.c.
   4·0 gm. of ammonium sulphate for every 10 c.c. of half-saturated solution.
It is better to weigh out the requisite amount of salt than to add it until no more dissolves, as an excess is in this way avoided and does not interfere with further operations.

The glutelins are insoluble in water and alcohol, but are soluble in very dilute alkali, or acid.

These proteins are considered in further detail with the other plant proteins (p. 439).

V. THE SCLEROPROTEINS.

The scleroproteins constitute the greater part of the skeletal structures of animal organisms. Just as their origin is very various, so also are their properties, but usually they are proteins insoluble in most reagents. They may be divided into several groups.

Keratins.

The keratins form the hard structure of hair, nails, feathers, horn, tortoise-shell, whalebone, etc.

The finely chopped material is boiled with alcohol, ether, and water. Fats, salts, and other soluble constituents are thus removed ; other proteins which may be present are removed by digestion with pepsin and trypsin. The insoluble residue is washed with water, alcohol, and ether and dried.

The keratins, when they have been dried, are hygroscopic substances and swell up slightly in water. They are insoluble in all reagents, but when boiled with acids and alkalies they dissolve and are converted into derivatives. Hydrogen sulphide and mercaptan are given off when they are boiled with acids. They are characterised chiefly by their high sulphur content, from 2 per cent. in pig's hoof to 15 per cent. in human hair.

They give most of the colour reactions for proteins (p. 371).

Egg-membranes.

The materials surrounding the eggs of birds, turtles, and fish are grouped together as a special group of scleroproteins, but they are closely related to the keratins. The hard material, koilin, in birds' crops may be included here.

The membrane is treated for several days with dilute caustic soda (0·1 per cent.), washed with water and soaked in dilute hydrochloric acid for several days to remove any inorganic matter, or gelatinous matter. The material is washed with cold water, boiling water, hot dilute acetic acid and boiled with alcohol and ether.
They are insoluble substances resembling the keratins ; they give most of the colour reactions of proteins, but generally contain less sulphur than the keratins.

Elastin.

Elastin is the constituent of elastic tissue and is especially abundant in the ligamentum nuchæ. The skeletal structure of the eggs of several fish and reptiles is said to consist of elastin.

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Ligamentum nuchæ is washed with water, treated for several days with fresh portions of half-saturated lime water to remove mucoids, boiled with 10 per cent. acetic acid, treated with cold 5 per cent. hydrochloric acid, again boiled with acetic acid and treated with hydrochloric acid, washed with water and boiled with alcohol and ether.

Elastin, as thus prepared, has a yellowish colour. It can be ground into a powder. The finely powdered substance dissolves slowly in cold ·2 per cent. hydrochloric acid, or 1 per cent. potash, on warming. By stronger acids, it is decomposed and forms derivatives.

It gives most of the colour reactions for proteins, but not the sulphur test.

Collagen.

Collagen forms the greater part of the ground substance surrounding the connective tissue cells in connective tissue, of the corpuscles in bone and of tendon; it also forms part of the substance of cartilage, cornea, and fish scales.

Bones are treated (1) with dilute hydrochloric acid to remove inorganic calcium salts, (2) with dilute alkali to remove organic matter.

Tendons are digested for several days with trypsin to remove proteins and washed with water.

Collagen is a colourless material which swells up in cold water, in dilute acids, and dilute alkalies. It is insoluble in organic solvents; it dissolves with swelling in strong caustic alkalies, but not in carbonates. It dissolves in pepsin solutions, but not in trypsin solutions, unless the collagen has been previously heated with water to 70°, or treated with acid. It is changed into derivatives when it is dissolved. It is converted by tannic acid into a form of leather, undergoing shrinkage. It slowly dissolves, on boiling with water, with evolution of ammonia and conversion into gelatin.

Gelatin.

Gelatin is formed by boiling collagen with water. The collagen of fish is the most easily converted into gelatin; that from older animals with greater difficulty than from young. The gelatin is obtained from the solution by evaporation and is generally procurable in the form of sheets.

Commercial gelatin may be purified by soaking it for several days in (1) water containing ether, (2) several weeks in changes of dilute sodium hydroxide, (3) very dilute acetic acid, (4) water. It is hardened with alcohol, dissolved in hot water and precipitated with alcohol. The precipitate is dried with alcohol and ether and placed in a desiccator over sulphuric acid.

Gelatin swells up in cold water, but does not dissolve. It dissolves in hot water and solutions above about 1 per cent. set to a jelly on cooling and redissolve on heating. The heating cannot be repeated very often, as the gelatin is hydrolysed and the solution no longer sets to a jelly on cooling. It dissolves very slightly in dilute alkali and is insoluble in alcohol.

*   Of the general reactions for proteins the xanthoproteic, Millon, sulphur, and Adamkiewicz' reactions are very faint.   Gelatin, therefore, does not contain those amino acids which are the cause of the reaction. It is not precipitated by concentrated mineral acids.

*   Mercuric chloride does not precipitate gelatin in neutral solution, but it precipitates it in presence of hydrochloric acid.

Hydroferrocyanic acid precipitates it only under certain conditions. Tannic acid gives a copious and voluminous precipitate.

Gelatin is not precipitated by saturation with sodium chloride, but comes down on acidifying.

Gelatin is precipitated from solution by saturation with magnesium sulphate, or by half-saturation with ammonium sulphate.

VI. PHOSPHOPROTEINS.

The phosphoproteins constitute the greater part of the protein present in the food-stuffs of young mammals and of embryo birds.   They are present in milk, in the eggs of birds, and the eggs of frogs and fish. Their characteristic property is that they contain phosphorus to the extent of nearly 1 per cent.   In this respect they resemble the nucleo-proteins and were formerly termed nucleoalbumins, but they differ from nucleoproteins in that the phosphorus is probably in combination with one of the amino acids ; in the nucleoproteins the phosphorus is present in the nucleic acid with which the protein is combined.

The chief phosphoproteins are caseinogen in milk and vitellin in egg-yolk (see later).

VII. NUCLEOPROTEINS.

The nucleoproteins are the constituents of cell nuclei and are conse-quently widely distributed.   A nucleoprotein has been prepared from almost every organ, but only a few have been at all well investigated. They are made up of varying amounts of protein and nucleic acid and are best considered as salts of protein with nucleic acid in different pro-portions, in the same way as the tribasic phosphoric acid can form three series of salts with bases.   The combination of the nucleic acid and the protein is very unstable, thus favouring the idea that they are salts.   The two components are easily separated by the action of alkali and the protein can be precipitated by alkaloidal reagents, leaving the nucleic acid in solution.   The substance in combination with protein is termed a prosthetic group.   The prosthetic group is nucleic acid.

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Two varieties of nucleoprotein can be distinguished :—

α-Nucleoproteins.

These are obtained when a tissue is treated with cold water, or cold 0·95 per cent. salt solution, and the milky solution after filtration acidified with acetic acid.

β-Nucleoproteins.

β-Nucleoproteins are obtained by boiling a tissue with water, so as to coagulate most of the proteins, and adding acetic acid to the clear filtrate. They contain less protein than the α-nucleoproteins and the prosthetic group is different. The guanylic acid of the pancreas is the best-known example of a β-nucleoprotein. The prosthetic group is a mononucleotide and contains a pentose in its molecule ; it is consequently a constituent of a plant nucleic acid (p. 325).

Jones considers that the β-nucleoproteins are not constituents of the cell nuclei, but that they are present in the contents of the cell having entered the tissue with the food.

Nucleins.

Nucleoproteins on digestion with pepsin are not completely hydro-lysed, i.e. the protein portion is not completely separated. A residue remains which is insoluble in the pepsin solution and consists of pro-tein combined with nucleic acid. This residue is termed nuclein. Nucleins are the insoluble residues (excluding remainders of sclero-proteins) which remain after tissues have been digested with pepsin. These residues frequently contain iron. The nuclein remaining when egg-yolk is digested with pepsin has been called hæmatogen on account of its iron content. It arises from the cell nuclei of the germinal layer.

Nucleoproteins and nucleins are insoluble in water and very dilute acids, but they dissolve in dilute alkali and stronger acids. One of their features is the presence of phosphorus and frequently the presence of phosphorus in a protein precipitate whether organic, or inorganic, has sufficed to place it in the class of nucleoproteins. The only character-istic of a nucleoprotein is the presence of nucleic acid and the formation of purine and pyrimidine bases on hydrolysis. Nucleoproteins may be distinguished from phosphoproteins by the action of alkali ; the former are stable and do not yield phosphoric acid on hydrolysis by alkali.

VIII. MUCOPROTEINS.

Most tissues of the animal body contain proteins which belong to the group of mucoproteins, which comprises the so-called glucoproteins, the mucins, and mucoids.

The mucoproteins are characterised by the formation of a reducing carbohydrate on hydrolysis.

They are conjugated proteins, consisting of

                  mucoitin sulphuric acid ⎫
or   chondroitin sulphuric acid ⎭ in combination with protein.

Like the nucleoproteins, their great number and variety depends upon the variety of the protein part of the complex molecule.

Mucoitin sulphuric acid is composed of :—

Sulphuric acid, acetic acid, chitosamine (2-glucosamine) and glycuronic acid.

Chondroitin sulphuric acid is composed of :—

Sulphuric acid, acetic acid, chondrosamine (2-galactosamine) and glycuronic acid.

The only difference between these compounds is thus in the nature of the hexosamine.

Levene gives the following formulæ to these compounds :—
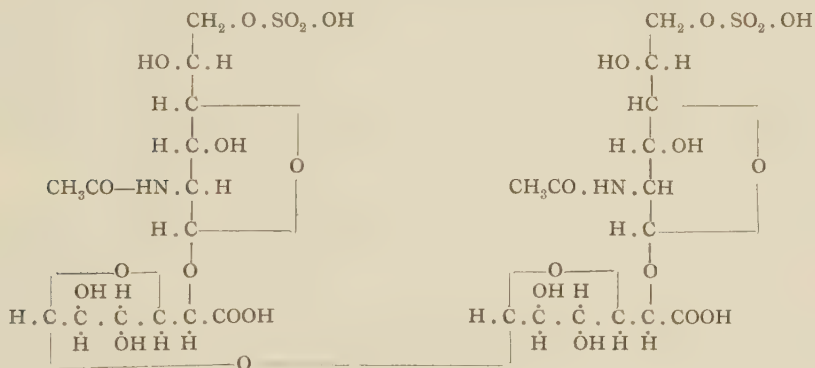
Chondroitin sulphuric acid.
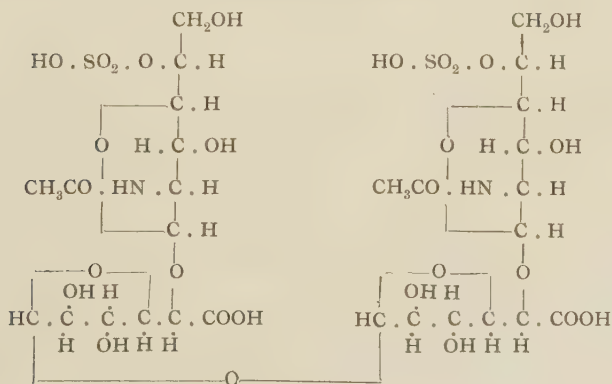
Mucoitin sulphuric acid.

The mucoproteins containing chondroitin sulphuric acid (chondro-proteins) are present in cartilage, tendon, aorta, sclera.

The mucoproteins containing mucoitin sulphuric acid are much more widely distributed and seem to be of two kinds :—

A. Funis, humor vitreous, and cornea mucins.

B. Gastric mucosa mucin, serum mucoid, ovomucoid.

The mucins in skin, submaxillary gland, urine, spleen, and other organs, are still unclassified.

Three kinds of mucoproteins can be distinguished :—

(1) *The Mucins.*—These are soluble in water and dilute alkali, but insoluble in excess of acetic acid and very dilute hydrochloric acid ; they give their solutions a slimy, or gummy, appearance and are pre-cipitated in sticky strands of material.

(2) *The Pseudomucins.*—These are soluble in water and dilute alkali, but unlike mucins are soluble in acetic acid.   Their solutions are slimy and they are precipitated by alcohol in the form of strands.

(3) *The Mucoids.*—These are soluble in water, dilute acids, and alkalies ; their solutions are not slimy ; on evaporation, they leave brownish membranes and they are precipitated by alcohol as white powders.   Most of the proteins belonging to this group have not been extensively studied and they deserve further investigation.

Mucin of the Submaxillary Gland.

Preparation.

The minced gland is treated with water.   The liquid is poured off and con-centrated hydrochloric acid is added until ·15 per cent. is present.   The mucin, which is precipitated, dissolves on stirring, but is thrown down by adding 2-3 volumes of water.   The liquid is poured, or strained, off; the precipitate is dissolved in ·15 per cent. hydrochloric acid and thrown down by adding water. This procedure is repeated.   The precipitate is washed with water, alcohol, and ether and dried.

Properties.

Submaxillary mucin forms an almost colourless powder which has an acid reaction.   It is not soluble in water, but dissolves on adding a trace of alkali, giving a slimy solution.   The solution is not coagulated by boiling, but is precipitated by acetic acid.   The precipitate is insoluble in excess of acetic acid and has the appearance of gelatinous strings which collect upon a glass rod when the precipitate is stirred.   The solution gives most of the colour reactions for proteins ; it is precipitated by alcohol in the presence of neutral salts and· also by heavy metals.   A solution containing salts is not precipitated by small quantities of acetic acid, nor by hydroferrocyanic acid, but it is precipitated by tannic acid.   Mucin is digested by pepsin and trypsin ; it is decomposed by dilute alkali giving gummy solutions and it is converted into a proteose by boiling with water at 110-150° with separation of a reducing carbohydrate.

20 per cent. of reducing carbohydrate calculated as glucose, together with acetic acid, is formed by hydrolysis on boiling for 3 hours with 3 per cent. hydrochloric acid.

Saliva.

Presence of Mucin.

* About 10-15 c.c. of saliva are collected in a beaker. Its reaction is faintly alkaline, but may be neutral, or slightly acid, at first from bacterial decomposition in the mouth.

* On adding acetic acid, the mucin is precipitated and is insoluble in excess of acetic acid ; on stirring the liquid, the mucin collects together on the rod and may thus be removed. The remaining liquid contains only traces of protein as shown by e.g. Millon's reagent.

* The precipitate on solution in dilute alkali carbonate will be found to give most of the general reactions for proteins. It is also soluble in ·1 per cent. hydrochloric acid.

* On boiling the solution with dilute hydrochloric acid for about 5 minutes, neutralising and testing with Fehling's solution, a small quantity of cuprous oxide will settle out on standing.

Presence of Thiocyanic Acid.

* Saliva generally contains small quantities of thiocyanic acid. This
· is shown by treating it with a drop of ferric chloride solution. A red colour is produced. The red colour is discharged by a drop of mercuric chloride solution. (Smokers' saliva generally contains more thiocyanic acid than that of non-smokers.)

IX. CHROMOPROTEINS.

The only well-defined member of this group of conjugated proteins is hæmoglobin, which is composed of the coloured substance hæmatin, or hæmochromogen, and the protein globin. The substance hæmo-cyanin, which is found in the blood of molluscs and crustacea, has been regarded as a similar conjugated protein, but more recent work does not confirm the older idea. Hæmocyanin contains copper in the place of iron in the coloured moiety of its molecule.

Hæmoglobin.

Hæmoglobin is present in the blood of all vertebrate animals and of some invertebrates. It is found either in solution in the liquid, or more usually, is contained in the red blood corpuscles, from which it can easily be separated. It is present in muscular and nervous tissue and under pathological conditions appears in the urine and fæces.

The hæmatin portion of hæmoglobin seems to be the same in all animals, but the protein portion may be different.

The hæmoglobin in the red blood corpuscles of the higher animals has been chiefly investigated. The red blood corpuscles can be separated from blood which has been prevented from clotting, e.g. by oxalates, or from defibrinated blood by centrifugalisation. Most of the properties and reactions of hæmoglobin can be easily studied directly with blood, or defibrinated blood, so that its isolation is not essential. (See later.)

CHAPTER XLV.

MILK.

MILK contains three proteins, caseinogen (or casein), lactoglobulin, lactalbumin, the carbohydrate lactose, butter fat, together with small amounts of lecithin and a yellow pigment, salts, chiefly calcium phosphate, and also in small quantities citric acid, creatine, allantoin. The milk of different animals has not the same percentage composition :—

	Human.	Cow.	Goat.	Mare.	Ass.	Whale.	Elephant.
Fat	3·5	3·5	4·3	1·1	1·6	19·4	19·6
Protein	1·5	3·5	4·6	1·9	2·2	9·5	3·1
Lactose	6·8	4·8	4·0	6·6	6·1	0	8·8
Ash	0·2	0·7	0·6	0·3	0·5	1·0	0·5

Cow's milk and goat's milk are very similar.   Human milk contains less protein and more lactose.   Mare's and ass' milk most nearly approach human milk in composition.   The other milks contain nearly 5 times as much fat and differ considerably.

Cow's Milk.

Appearance.

Milk is a white, or pale yellow, fluid which is opaque except in thin layers.   The peculiar non-transparent appearance is due partly to an emulsion of finely divided fat particles and partly to the opalescence of the calcium salt of caseinogen.

* The *fat particles* of varying size are visible under the microscope and some of them exhibit Brownian movement.

* The *specific gravity* of milk varies from 1028-1035.   It is usually taken with a lactometer, a specially graduated and delicate hydrometer. It rises gradually for some time after it has been drawn and the specific gravity should not be taken till after 5 hours.   In mixing milks the formation of air bubbles must be carefully avoided, as they are held rather tenaciously and cause errors in the determination of the specific gravity owing to the removal of the fat, the lightest constituent, which

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floats to the surface. Skimmed milk has a higher specific gravity, from 1033-1037.

The *reaction* of milk to litmus is amphoteric, but it is generally alkaline to red litmus paper.

The Effect of Heat.—No coagulation of proteins occurs on boiling milk, but a scum forms on hot milk on standing. This is due to evaporation on the surface, a layer of the dried constituents forming there and separating out. It forms each time milk is boiled and allowed to cool. The coagulated proteins will be present in the first scum.

Differentiation between Fresh and Boiled Milk.

Fresh milk gives *the Guaiac reaction.* Some tincture of guaiacum is added to a little fresh milk and a little hydrogen peroxide. On mixing and allowing to stand, it gradually becomes blue owing to the oxidation of the guaiacol in the tincture by an oxidising enzyme, or oxidase, in the milk. Instead of hydrogen peroxide, old oil of turpentine, which contains a peroxide, may be used. The reaction generally succeeds better with this than with the hydrogen peroxide.

Boiled milk does not give this reaction, as the oxidase is destroyed by heating.

Separation of the Fat.

The fat of milk gradually rises to the surface on standing, forming cream, but it is generally separated by centrifugalisation. The fat may also be extracted by shaking some milk with twice its volume of ether. The ether deposits the fat on evaporation. After removal of the fat by ether, the opacity of milk is hardly altered and is due to the opalescence of the calcium salt of caseinogen.

If milk be treated with a little caustic soda and shaken with ether, the aqueous solution is translucent, as the sodium salt of caseinogen is formed, which does not give an opalescent solution.

Butter.

Butter is obtained from cream by churning. It consists of the triglycerides of palmitic, stearic, and oleic acids like other fats, but contains in addition the triglycerides of butyric and caproic acids (tributyrin, tricaproin). Recent work by Caldwell and Hurtley points to the absence of tributyrin in butter. When it becomes rancid by the action of bacteria—which contain the enzyme, lipase—the smell of butyric acid and caproic acid is noticeable. The presence of these lower volatile fatty acids constitutes the chief difference between butter and ordinary fat. The butyric acid probably arises from the glutamic acid of the caseinogen which is converted by bacteria into butyric acid. Butter

usually contains some milk and therefore also caseinogen; hence the origin of the butyric acid.

Margarine is prepared from animal fat, or from vegetable fats. These fats are generally oils, so that they are hardened. This is effected by hydrogenisation, i.e. reducing unsaturated fatty acid to the saturated. Butter and milk are frequently added to margarine.

Caseinogen and other Proteins.

*   10-20 c.c. of milk diluted with 3 volumes of water are gradually acidified with dilute acetic acid, avoiding excess; a flocculent precipitate of caseinogin and fat is formed.

The filtrate, on nearly neutralising with soda and boiling, gives a precipitate of lactoglobulin and lactalbumin.

Presence of Lactose and Phosphates.

*   The filtrate, after the separation of the coagulable proteins, will show the presence of a reducing carbohydrate; its nature can be determined by preparing the osazone with phenylhydrazine and acetic acid.

*   The same filtrate will give the reactions for phosphate with nitric acid and ammonium molybdate, or magnesia mixture and ammonia.

*   Milk itself turns yellow, then brown, on heating with dilute caustic soda, due to the action of alkali on lactose.

Separation of the Proteins.

*   Saturation of milk with sodium chloride precipitates the calcium salt of caseinogen together with the fat.

The filtrate is saturated with magnesium sulphate. Lactoglobulin is precipitated.

It is purified by dissolving in water and again precipitated and the process is repeated. The precipitate is dissolved in water and dialysed to remove salts, or coagulated by heat in acid solution.

Lactoglobulin closely resembles serum globulin. Crowther and Raistrick's analysis of lactoglobulin points to its identity with serum globulin.

Lactalbumin is obtained from the filtrate by acidifying with acetic acid, so that the content of acid is about 1 per cent.

The precipitate is filtered off, pressed out, and dissolved in water; the solution is neutralised and dialysed; the lactalbumin is obtained on evaporation *in vacuo*, or as coagulated protein by heat coagulation, or by precipitation with alcohol.

Lactalbumin is very similar to serum albumin, but differs in rotation and percentage composition. It has been obtained in a crystalline state in the same way as serum albumin. It behaves like serum albumin in other respects.

Human Milk.

In appearance human milk resembles cow's milk, but it has a different composition as shown in the table on p. 425. The amount of phosphates in human milk is also less than in cow's milk.

Not only is there a difference in composition, but also the caseinogen of human milk appears to be different. It is more difficult to precipitate this caseinogen with acid.

The preparation of caseinogen from human milk is best effected by freezing the milk for 2-3 hours, after diluting with 5 volumes of water, and adding 60-80 c.c. of ·1N acetic acid and subsequently shaking and warming to 40° for a few minutes.

Human milk does not always clot with rennet (p. 430). This depends on the smaller quantity of calcium salts in human milk. The clot is also usually not so firm as the clot of cow's milk and the calcium caseate separates in flakes.

Humanised Milk.

Cow's milk can be altered in composition so as to approach, or be the same as, that of human milk. Dilution of cow's milk reduces the amount of protein; lactose and fat are then added to bring up the content of these constituents. Humanised milk is most easily and best prepared by adding an equal volume of whey to cow's milk and then lactose and fat. If, in preparing the whey, the milk be continually stirred, the caseinogen is separated and the fat remains in suspension in the liquid.

Caseinogen.

Not only is caseinogen the chief protein present in milk, forming as it does about 80 per cent., but also it is of great value as a food-stuff. Cheese consists mainly of casein and fat. There are numerous preparations of dried milk and commercial preparations, such as plasmon, sanatogen, nutrose, and many others.

These food-stuffs are generally fine powders soluble in water; they are frequently mixed with carbohydrates and medicinal substances, such as sodium glycerophosphate in sanatogen. The caseinogen is usually in the form of a sodium, potassium, or ammonium salt prepared by treatment with sodium bicarbonate, sodium citrate, sodium phosphate. The amount of protein in these substances is from 20-95 per cent.

It is used commercially for many purposes, e.g. in making painting materials, in making adhesives, in making artificial ivory, celluloid, bone, buttons; in waterproofing paper materials; in making fireproof materials

when mixed with asbestos; in glazing, in solidifying oils, in making soaps.

Preparation.

Commercial caseinogen is prepared by treating skimmed milk with dilute sulphuric, or hydrochloric, acid. The precipitate of caseinogen is filtered off and dried; the dry preparation contains about 10 per cent. of water.

Pure caseinogen is prepared by acidifying skimmed milk which has been diluted with water, washing the precipitate with water, dissolving it in dilute alkali, reprecipitating and repeating this procedure several times. The precipitate is finally treated with alcohol and ether, to remove fat, and dried.

Properties.

* Caseinogen forms a white granular powder, which is insoluble in water; it is insoluble in dilute acids in the cold, but on warming it dissolves slowly, undergoing decomposition into derivatives. It dissolves in dilute alkalies forming salts; these are best made by rubbing the caseinogen in a mortar with small quantities of the alkali, or alkali carbonate, in the presence of a drop of phenolphthalein, more alkali being added only when the pink colour disappears. 1 gm. of caseinogen dissolves in 8 c.c. of ·1N alkali. On rubbing with carbonates, carbon dioxide is evolved.

Caseinogen forms two series of salts; those with calcium oxide contain respectively 1·5 and 2·5 per cent. of calcium oxide. The neutral salt containing 1·5 per cent. of CaO is neutral to litmus; the basic salt containing 2·5 per cent. of CaO is neutral to phenolphthalein. A solution of caseinogen in lime-water when made neutral to phenolphthalein is milky in appearance, but when neutralised to litmus the milky appearance is much more distinct.

* A neutral solution of caseinogen in caustic alkali gives most of the general reactions for proteins.

The presence of phosphorus in caseinogen is shown by incinerating, or oxidising, it and testing for phosphates (p. 21).

* The phosphorus in caseinogen and phosphoproteins is separated by the action of dilute alkali. On warming caseinogen with caustic alkali of about 1 per cent. strength for five minutes in a boiling water-bath, acidifying and filtering, the filtrate will show the presence of inorganic phosphates when tested with nitric acid and ammonium molybdate.

The Action of Rennin upon Caseinogen.

*   The most characteristic property of caseinogen is its conversion into casein, or paracasein, by the action of the enzyme, rennin or chymosin.

Rennin is prepared by extracting the fourth stomach (rennet) of the calf with salt solution, or glycerol, and can be obtained commercially either in solution, or in the form of powder or tablets.

The conversion is most noticeable with the soluble calcium salt of caseinogen as present in milk which is converted into the insoluble calcium salt of casein; the milk only clots in the presence of calcium salts.

The conversion takes place more rapidly in the presence of a trace of acid, or acid salts, but is prevented by alkali. The presence of calcium salts is essential to the formation of a clot. Boiled milk does not clot with rennin since soluble calcium salts (bicarbonates, acid phosphates) are made insoluble (carbonates, phosphates), but it clots if soluble calcium salts, or a few drops of acid, be added. The change seems to take place in three stages: (1) the conversion of calcium caseinogenate into calcium caseate; (2) the formation of soluble calcium salts from insoluble salts; (3) the change in viscosity and clotting of the calcium caseate.

*   The factors concerned in the clotting of milk are illustrated by the following experiments. The various mixtures of milk and rennet are placed in a water-bath at 40° and the time taken to produce coagulation is noted :—

	Time of Coagulation.
(1) 5 c.c. of milk + 2 c.c. of rennet extract	+
(2) 5 c.c. of milk + 2 c.c. of boiled rennet extract	o
(3) 5 c.c. of milk + 1 or 2 drops dilute acetic acid + 2 c.c. of rennet extract (More acetic acid precipitates caseinogen)	+
(4) 5 c.c. of milk + 2 c.c. dil. Na_2CO_3 solution + 2 c.c. rennet extract .	o
(5) 5 c.c. of milk + 1 c.c. of potassium oxalate solution + 2 c.c. of rennet extract	o
(6) 5 c.c. of milk + 1 c.c. of potassium oxalate solution + 2 c.c. of rennet extract for 10 minutes. The rennet is now destroyed by boiling, the solution cooled and 1 c.c. of $CaCl_2$ solution added; clotting, or precipitation, occurs	+
(7) 5 c.c. of cold boiled milk + 2 c.c. of rennet extract	o
(8) 5 c.c. of cold boiled milk + 1 c.c. of calcium chloride solution + 2 c.c. of rennet extract	+
(9) 5 c.c. of cold boiled milk + 2 or 3 drops of dilute acetic acid + 2 c.c. of rennet extract . . . . (The acid produces soluble calcium salts and accelerates the clotting)	+

The coagulation of milk takes place normally in digestion in the stomach and is usually attributed to the presence of the enzyme, rennin, but Pavloff and other workers consider that the clotting of milk is pro-

duced by the action of pepsin in neutral, or very faintly acid, solution. The identity of the two enzymes, pepsin and rennin, is emphasised by the fact that the proteoclastic enzymes of plants, such as occur in pineapple juice, clot milk, if their solutions be nearly neutralised. Trypsin in very small amounts will also clot milk.

If the change of calcium caseinogenate into calcium caseate is a stage of pepsin digestion, casein may represent the stage of metaprotein ; the calcium salt of it is insoluble and is therefore precipitated.

Cheese.

The action of rennin upon milk is used in the preparation of junket and cheese. Extract of rennet is added to milk, or skimmed milk, and the mixture kept in a warm place. It clots and forms junket. On standing, the clot contracts and a clear liquid, termed whey, exudes. By cutting the clot into cubes the whey oozes out more rapidly, and as it oozes out the cubes are stirred, cut smaller and gradually piled one upon another, so that they press out the remainder of the whey. The cubes gradually form a mass which is again cut up and the process continued till the mass is sufficiently dry. Salt is added and the mass is pressed out so that it forms a solid lump. It is allowed to stand and ripen, a process which is due to enzyme action. These are hard cheeses. By the action of moulds and bacteria they become green.

Soft cheeses are prepared in the same way, but the whey is not removed by cutting the curd ; the clot is allowed to contract and most of the whey may be pressed out, but the soft cheese always contains some whey. It ripens more rapidly than hard cheese, and the ripening is due to the action of bacteria.

The whey, which has oozed out, contains the lactose, phosphates, and soluble proteins and is used for preparing milk sugar.

CHAPTER XLVI.

THE PROTEINS OF EGGS, MUSCLE, AND OTHER ANIMAL TISSUES.

EGG-WHITE.

WHITE of egg is a pale yellow fluid contained in a net-work of a fibrinous material. The net-work is broken up by beating the egg and the egg-white is obtained by straining through calico.

Egg-white has a faintly alkaline reaction to litmus and a specific gravity of 1·045. It contains 12 to 15 per cent. of solid matter, 85-88 per cent. being water.

The solid matter is almost entirely protein; there is about ·5 per cent. glucose, ·66 per cent. ash and traces of soaps, fat, and cholesterol.

Of the protein over 80 per cent. is albumin, 6·7 per cent. is globulin, 10 per cent. is ovomucoid, a glucoprotein (p. 420).

Globulin (Ovoglobulin).

The globulin is precipitated from egg-white, or a solution of egg-white, by saturation with sodium chloride, or magnesium sulphate, or by half-saturation with ammonium sulphate.

An equal volume of saturated ammonium sulphate solution is added slowly to egg-white, or a solution of egg-white in water, with constant stirring. After standing, the precipitate is filtered off, dissolved in water [1] and precipitated again with ammonium sulphate.

This process is repeated several times. The final solution is dialysed to remove salt and the protein can be obtained by evaporation of the solution at a low temperature.

In a *coagulated* state it is separated by acidifying and boiling the solution in water. A dry preparation is obtained by filtering off the coagulum, dehydrating with alcohol, washing with ether, and drying in the air.

Uncoagulated globulin forms an amorphous yellow mass, insoluble in water, but soluble in dilute salt solutions; it is precipitated from solution by saturation with sodium chloride, magnesium sulphate, or by half-saturation with ammonium sulphate.

[1] Sufficient salt is still present to make the solution a dilute salt solution.

The solution in salt solutions shows all the general reactions for proteins.

Coagulated globulin is an amorphous white powder which is insoluble in water and dilute salt solutions. It dissolves slowly, on warming, in dilute acids and alkalies, undergoing hydrolysis into derivatives (metaprotein).

A suspension in water will show most of the colour reactions for proteins.

This substance has been termed ovomucin by Osborne and Campbell. It is not certain if it is a single protein.

Albumin (Ovalbumin).

* Ovalbumin remains in solution after the globulin has been precipitated by half-saturation with ammonium sulphate.

The filtrate is saturated with finely powdered crystals of ammonium sulphate. The precipitate is dissolved in water and again precipitated with ammonium sulphate and the process is repeated several times. The last precipitate is dissolved in water and the solution is dialysed to remove the salt. The albumin is obtained on evaporation *in vacuo* at 40°.

* *Coagulated albumin* is obtained by acidifying the solution and boiling, washing the coagulum with water, dehydrating with alcohol, washing with ether, and drying by exposure to the air.

Crystalline Ovalbumin.

The albumin in egg-white is readily prepared in a crystalline form by the method of Hopkins. This consists in carefully adding acetic acid to egg-white treated with an equal volume of saturated ammonium sulphate solution and thus free from globulin. Thus, fresh egg-white is beaten into a froth with an exactly equal volume of saturated ammonium sulphate solution and the mixture is filtered after standing for some hours. 10 per cent. acetic acid is added to the filtrate from a burette with constant stirring until it becomes distinctly turbid. 1 c.c. of acetic acid is then added for every 100 c.c. of filtrate. An amorphous precipitate is first formed, but on standing it becomes crystalline, and with frequent shaking the whole of the ovalbumin crystallises in 5-6 hours. After 24 hours it is filtered off, washed with ammonium sulphate containing ·1 per cent. of acetic acid and dissolved in water (1 part in 10). Saturated ammonium sulphate solution is carefully added with gentle shaking until a permanent precipitate results, and then for every litre 2 c.c. more of ammonium sulphate. The ovalbumin crystallises out and is washed as before. The crystalline mass contains ammonium sulphate. A solution free from ammonium sulphate is obtained by dialysis, or as above either in the uncoagulated, or coagulated, condition. The yield is 50 gm. from 1000 c.c. of egg-white.

Conalbumin.

The whole of the ovalbumin can never be obtained in a crystalline condition. According to Osborne and Campbell only 50 per cent. of the albumin can be crystallised. The remainder is termed conalbumin. It is prepared from the filtrate by saturation with ammonium sulphate.

Uncoagulated ovalbumin is an amorphous mass of a yellowish colour, soluble in water and dilute salt solutions. It is not precipitated from solution by saturation with sodium chloride, magnesium sulphate, or half-saturation with ammonium sulphate, but is precipitated by complete saturation with ammonium sulphate.

A 2·5 per cent. solution in water coagulates at 60-64°; in 10 per cent. salt solution at 68-70°.

Conalbumin is very similar to ovalbumin, but it coagulates at a lower temperature and has a higher rotation.

Solutions of ovalbumin, crystalline ovalbumin, and conalbumin give all the general reactions for proteins.

The coagulated albumins are insoluble in water and salt solutions, but dissolve slowly in acid and alkali yielding solutions of derivatives (metaprotein).

Ovomucoid.

Ovomucoid is best obtained directly from white of egg. White of egg is poured into boiling water (5-10 volumes) faintly acidified with acetic acid (·1 per cent.). The albumin and globulin are coagulated and filtered off. The filtrate is evaporated down to a small volume and poured into 4-5 volumes of alcohol. Ovomucoid is precipitated. It is dissolved in warm water and reprecipitated with alcohol, washed with alcohol and ether and dried.

Ovomucoid forms a colourless amorphous powder, soluble in cold water. The aqueous solution, on evaporation, leaves a horny, transparent residue, insoluble in cold water. Solutions of ovomucoid are not precipitated by mercuric chloride, but they are precipitated by tannic acid and other alkaloidal reagents.

On boiling with acid, glucosamine is formed, as can be shown by heating with concentrated hydrochloric acid for 5 minutes, neutralising, and testing with Fehling's solution. The cuprous oxide is generally seen after the solution has stood so as to allow it to settle.

EGG-YOLK.

Egg-yolk contains fat, lecithine, lutein (the yellow colouring matter), cholesterol, the phosphoprotein vitellin, and small quantities of a co-agulable protein, livetin. The vitellin and lecithine appear to be in a kind of loose combination and this combination was formerly termed vitellin. It is preferable to term the protein vitellin and the combination lecitho-vitellin.

Lecitho-vitellin.

Egg-yolk is mixed with 10 per cent. sodium chloride solution and the mixture is extracted several times with ether. The salt solution is dialysed, or poured into 20 volumes of water. The precipitate is purified by solution in sodium chloride and precipitation with water.

A solution of lecitho-vitellin in 10 per cent. sodium chloride, prepared from egg-yolk as above, coagulates on heating at 70-75°. The compound in solution behaves like a globulin in being insoluble in water and in being precipitated by saturation with magnesium sulphate, or half-saturation with ammonium sulphate. It gradually breaks up into lecithine and vitellin by repetition of precipitations, or on long contact with water. It gives the colour reactions and most of the precipitation reactions for proteins. It is converted by alcohol into lecithine and vitellin.

Livetin is contained in the water into which the egg-yolk solution in sodium chloride is poured. It coagulates when the solution is acidified and boiled. It differs in this way from vitellin as well as in its phosphorus content.

Vitellin.

The precipitate of lecitho-vitellin is treated with warm alcohol and ether. The lecithine is dissolved and the vitellin remains.

Since the protein of egg-yolk consists mainly of vitellin, it can be prepared directly from dried yolk (commercial or from yolks spread out on a plate and allowed to dry in the air) by extracting with cold alcohol, hot alcohol and ether. The residue is vitellin, containing livetin and other constituents of yolk in smaller quantities.

Vitellin forms a granular powder, which is pale yellow to reddish-yellow in colour. It is insoluble in water and salt solutions; also in dilute acids; it dissolves slowly in stronger acids undergoing hydrolysis. It also dissolves slowly in dilute alkali and probably undergoes hydrolysis. It contains about 1 per cent. of phosphorus as shown by incineration, or other methods of oxidation (p. 21). The phosphorus is split off by warming with dilute alkali in the same way as from caseinogen (p. 429).

THE PROTEINS OF MUSCLE.

The solid matter of muscle consists essentially of proteins, the principal other constituents being fat, extractives (creatine, purines, and other nitrogenous substances) and lactic acid. Lean meat has the following average composition:—

Water 75

Solid 25 {
Protein 20
Extractives 3
Fat 1
Salts 1
}

Living muscle consists of a semi-fluid muscle plasma which is faintly alkaline in reaction to litmus. As the result of death the soluble proteins undergo clotting—*rigor mortis*—and become insoluble ; the reaction becomes acid due to the formation of lactic acid. The coagulation is accelerated by acids and by a rise of temperature and does not occur in weak alkaline solutions, or in the absence of salts.

During life, the coagulation change seems to be brought about by lactic acid, and the disappearance of the insoluble protein is owing to its re-solution by the lactic acid which also disappears and is again built up into the soluble protein.

The disappearance of *rigor mortis* after death is probably due to solution of the insoluble protein by a proteoclastic enzyme which converts it into metaprotein and other derivatives.

Two soluble proteins—only one according to Mellanby[1]—are present in living muscle :—(1) Paramyosinogen, (2) myosinogen in the proportions of one-fifth and four-fifths respectively. These are converted by clotting into myosin, the former directly, the latter through the stage of soluble myosin, thus

```
Paramyosinogen              → ⎫
   (or myosin)                 ⎬ Myosin
Myosinogen → Soluble myosin → ⎭  (or muscle fibrin)
   (or myogen)
```
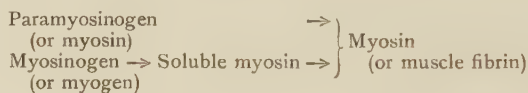
Paramyosinogen and Myosinogen.

*   Fresh veal, or the muscles of a rabbit freed from blood by perfusing the vessels through the aorta with ·9 per cent. sodium chloride solution, are chopped up finely and extracted with ·9 per cent. sodium chloride solution.

The extract is slightly acid due to the presence of lactic acid which can be tested for by Uffelmann's test, or Hopkins' test (p. 144).

The extract is treated with three-fourths of its volume of saturated ammonium sulphate. The paramyosinogen is precipitated. It is dissolved and reprecipitated several times, or separated out by dialysis. The protein can then be prepared as described under ovoglobulin and serum globulins.

The filtrate from the paramyosinogen is saturated with ammonium sulphate. The precipitate of myosinogen so formed is purified by solution and reprecipitation.

*   The residue after the above extraction of paramyosinogen and myosinogen is ground up with sand and treated with 5 volumes of

[1] J. Physiol., 1908, Proc.

10 or 15 per cent. ammonium chloride solution.   The liquid is strained from sand and insoluble protein.

On dialysis, it yields a precipitate of paramyosinogen, which is purified by re-solution in salt solution and dialysis.

The properties of paramyosinogen can be well seen with this extract :—

*   (1) Insolubility in water.   On pouring some of the solution into a large volume of water, it is precipitated : the liquid is decanted off. The remaining suspension is—

(2) Soluble in dilute sodium chloride and precipitated on saturating with sodium chloride.

(3) Soluble in dilute ammonium sulphate and precipitated by half-saturation with ammonium sulphate.

(4) Coagulated at about 47° when dissolved in dilute ammonium sulphate.

One of the chief differences between these proteins is their temperature of heat coagulation.   Paramyosinogen coagulates at 47°; myosinogen at 56°.

The extract obtained from fresh muscle, on heating, will coagulate at about 47° and after filtering off the flakes of protein it will coagulate again at about 56°.   (Coagulation may take place at about 40°. This is the coagulation temperature of soluble myosin formed from myosinogen.)

Paramyosinogen is a typical globulin; it is insoluble in water and is precipitated by half-saturation with ammonium sulphate.   Myosinogen is an atypical globulin; it is soluble in water and is only partially precipitated by half-saturation with ammonium sulphate.   It resembles an albumin very closely.

They give the general reactions for proteins.

Myosin.

Myosin is insoluble in water and dilute salt solutions and remains as a residue with the connective tissue.   It has the properties of a co-agulated protein and dissolves on warming in dilute acids and alkalies, giving solutions of the metaprotein, syntonin.

THE PROTEINS OF OTHER ANIMAL TISSUES.

Most tissues on treatment with salt solution yield an extract which contains proteins similar to those of muscle; further, a protein like paramyo-sinogen can be extracted with 10-15 per cent. ammonium sulphate solution. The bulk of the protein, as in the case of muscle, consists of coagulated protein, like myosin.

These proteins have been very little investigated except those in the thyroid and crystalline lens.

Thyreoglobulin.

Thyreoglobulin is a globulin of the thyroid gland which contains iodine in organic combination and to which the physiological action of the gland is said to be due.

Crystallin.

The crystalline lens of animals contains apparently two globulins which have been termed α- and β-crystallin.

CHAPTER XLVII.

THE PROTEINS OF PLANTS.

A. GLOBULINS OF LEGUMINOUS SEEDS.

The pea, horse bean, lentil, and vetch seem to contain the same principal protein, the globulin termed legumin. Another globulin, termed vicilin, is present in the first three seeds. These globulins are separated from one another by fractional precipitation with ammonium sulphate. Small quantities of the albumin, legumelin, are also found in these seeds.

Separation of the Proteins of a Leguminous Seed.

10 gm., or more, of pea flour are stirred up with twice the weight of 10 per cent. sodium chloride solution and allowed to stand for about an hour. The liquid is filtered from the insoluble residue, which consists mainly of starch, and saturated with ammonium sulphate crystals.[1] Legumin, vicilin, and legumelin are precipitated. The precipitate is filtered off and dissolved in dilute ammonium sulphate solution ($\frac{1}{100}$ saturated). Saturated ammonium sulphate solution is added in the proportion of 150 c.c. to 100 c.c. of solution ($\frac{6}{10}$ saturation). The legumin is precipitated; it is purified by repeating the process of precipitation with $\frac{6}{10}$ saturated ammonium sulphate and separated by dialysis. The filtrate from the legumin is saturated with ammonium sulphate. Vicilin and legumelin are precipitated. They are purified by solution, $\frac{6}{10}$ saturation and complete saturation with ammonium sulphate as above. They are separated from one another by dialysis. Vicilin is precipitated, legumelin is not precipitated. It is obtained after filtration of the vicilin, by heat coagulation, or by evaporation of the solution *in vacuo* at 40°.

B. GLOBULINS OF NUTS.

Nuts contain globulins as their principal protein. Many of these globulins have been obtained in a crystalline form. The amount of albumin in these seeds is very small.

Separation of Globulins of Nuts.

The ground-up nut is treated with petroleum ether to remove the oil, or fat.

The fat-free residue (10 gm. or more) is treated with twice its weight of 10 per cent. sodium chloride solution. The solution is filtered off and poured

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[1] 80 gm. per 100 c.c.

into a large volume of water, or dialysed. A precipitate of globulin is produced which gradually settles and is purified by solution in salt solution and precipitation with water. It is filtered off and washed with water.

The globulins of the oil seeds prepared as above are usually amorphous powders, insoluble in water but soluble in salt solutions. They have the general properties of a globulin and show the general reactions of proteins.

Preparation of Crystalline Globulins from Oil Seeds.

(a) *Excelsin from Brazil Nuts.*

The nut is ground up and freed from oil as described under edestin. The residue is extracted with 10 per cent. sodium chloride solution and the extract is dialysed. It is thus precipitated slowly from solution and is obtained in crystalline hexagonal plates.

(b) *Edestin from Hempseed.*

The seed (1 kilo) is crushed and pressed in a hydraulic press to remove the oil. The residue is treated with petroleum ether and again pressed. The fat-free residue is extracted with 1000 c.c. of 5 per cent. sodium chloride solution at 60° and the solution filtered through calico. On cooling, a precipitate settles out; it is filtered off, washed with water, and dissolved in 500 c.c. of 5 per cent. sodium chloride solution at 60°. On cooling, edestin separates in a crystalline form. It is filtered off, washed with salt solution, alcohol, and ether and dried in the air. About 100 gm. are obtained from 1000 gm. of hempseed.

A further quantity of edestin separates, if the filtrate be dialysed, but as spheroidal masses and not in the same crystalline state.[1]

C. GLIADINS AND GLUTELINS OF CEREALS.

The chief constituents of the seeds of cereals are starch and protein. Small quantities of fat and salts are also present.

The percentage composition of the protein matter of the flour of cereals is the following :—

	Wheat.	Barley.	Maize.	Oats.	Rice.	Rye.
Total protein	10	11	8·6		7·0	8·5
Gliadin	4·25	4·0	5·0	1·25	0	4·0
Glutelin	4·0	4·5	3·1			2·5
Globulin	·6 }	2·3	·45	1·5		2·0
Albumin	·3 }					

The mixture of glutelin and gliadin in roughly equal proportions is known as gluten.

The chief protein constituents form a distinct group which is not represented in other plants, nor in animals. They are therefore known as gliadins and glutelins.

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[1] See also Reeves, Biochem. J., 1915, **9**, 508.

The albumin, termed leucosin, is present in wheat in small quantities. Similar albumins are present in other cereals. They form 0·2-2 per cent. of the total protein.

Gluten.

About 20 gm. of flour is made into a dough with 8-10 c.c. of water and the dough is allowed to stand for 15-60 minutes. It is washed by kneading in a piece of muslin under running water. The washing is continued until no more starch can be removed. The remainder has a sticky elastic consistency and is of a grey to pale yellow colour. When it is dried, the gluten forms a brittle mass, like glue, and is yellow-brown in colour. It is contaminated with the fat of the grain and some starch.

The formation of gluten is due to the gliadin, which makes a sticky mass with water and binds together the particles of glutelin. The elastic properties of gluten depend mainly upon the proportion of salts present in the flour, especially phosphates. Rye does not form gluten, but contains a large proportion of a gummy polysaccharide.

Gliadin.

Gliadin is prepared from flour by extracting it with hot alcohol (74 per cent. by volume). The alcohol extract is concentrated *in vacuo* and poured into absolute alcohol. The precipitate may be dissolved in dilute alcohol and poured into absolute alcohol.

Gliadin, prepared as above, forms an amorphous powder, but, if prepared by evaporation of the dilute alcohol, it forms a yellow-brown sticky mass which dries like glue.

Gliadin is insoluble in water and in absolute alcohol, but dissolves in dilute alcohol of 50-70 per cent. It is also insoluble in dilute salt solutions. It dissolves slowly in warm acids and alkalies and is converted into derivatives.

It gives most of the general reactions for proteins.

Besides its peculiar solubilities gliadin also differs considerably in composition from other proteins: it contains a large amount of proline and also amide groups—hence gliadins have been termed prolamins. They also contain a large amount of glutamic acid.

Wheat Glutelin, or Glutenin.

Glutenin is most easily prepared from gluten. The gluten is boiled several times with alcohol and the residue dissolved in 0·2 per cent. potassium hydroxide. On neutralisation of the solution with acetic acid, the glutenin is precipitated and may be purified by repeating the solution in alkali and precipitation with acetic acid. The precipitate is washed and dried with alcohol and ether.

Glutenin forms an amorphous powder which is insoluble in water and in salt solutions, but soluble in ·2 per cent. acid, or alkali. It is converted

into derivatives by stronger acids and alkalies.   It gives most of the general reactions for proteins.

Globulin and Albumin from Cereals.

These proteins are present only in small quantities in the flour, but the germ contains up to 10 per cent. of coagulable proteins.

The germ is extracted with water; sufficient salt is present to dissolve some of the globulin, or the germ is extracted with 10 per cent. salt solution. The proteins are precipitated by saturating the extract with ammonium sulphate.   The precipitate is dissolved in water, or salt solution, and dialysed. The globulin is precipitated and purified by again dissolving and dialysing. The albumin (leucosin of wheat) in the solution is obtained by heat coagula tion.   The two proteins have the general properties of globulins and albumins.

CHAPTER XLVIII.

DIGESTION.

THE three classes of organic compounds, the fats, the carbohydrates, and the proteins, taken in as food are hydrolysed by enzymes into their constituents before they can pass through the wall of the alimentary canal and can be assimilated.

I. SALIVA.

The first digestion of food occurs in the mouth by the action of the saliva, the secretion of the salivary glands. The saliva contains the enzyme, diastase, or amylase, which hydrolyses starch (and glycogen) converting it into dextrin and maltose. In the mouth, however, very little enzyme action takes place, the food being only moistened by the saliva and swallowed. The action occurs in the stomach, where the food is in the form of a mass in the fundus; here only the exterior of the mass is in contact with the hydrochloric acid of the gastric juice, which inhibits the action of the diastase; the starch in the interior of the mass is slowly digested.

Action of Salivary Diastase.

A solution of this diastase is prepared by rinsing out the mouth two or three times with 20 c.c. of distilled water, warmed to 40°, for 1-2 minutes. The water is collected in a beaker and filtered.

The presence of diastase in the solution is demonstrated in the same way as for malt diastase (p. 406).

A 1-2 per cent. solution of soluble starch, or starch paste, is prepared as substrate. The presence of diastase is shown by the disappearance of starch and the appearance of erythrodextrin, achroodextrin and maltose; thus :—

A series of drops of iodine solution are placed upon a porcelain plate, or upon a glass plate on white paper.

5 c.c. of starch solution are placed in each of two test tubes, 5 c.c. of diastase solution is placed in the one and 5 c.c. of boiled diastase

solution in the other.   The second tube acts as a control.[1]   The test tubes are placed in a bath at 40°, as the hydrolysis proceeds more rapidly at this temperature.

A drop is removed from each solution immediately after the mixtures have been made and placed against an iodine drop.

At intervals of half a minute, or a minute, drops are removed from each test tube and placed against another iodine drop.   The blue colour given by the control tube is given each time the test is made, but the colour becomes reddish-brown in the case of the other tube and finally no colour at all is given.   Achroodextrin and maltose have been formed.   Each test tube, after about 5 minutes, is tested with Fehling's solution.   The control tube shows no reduction, but the presence of maltose in the other tube is shown by a marked reduction.

*   *Effect of Temperature, 0°, 45°, 100°, upon Diastase.*

The same experiment is performed with two test tubes containing 5 c.c. of starch solution and 5 c.c. of diastase solution, but one of them is placed in cold water, or better, ice, and the second in a water-bath at 45°.   The times are noted when the various colours are given and when no colour is given with the iodine drops.

The hydrolysis takes place more slowly in the tube kept at the lower temperature.

*   *Effect of Acid, Alkali, and Salt upon Diastase.*

5 c.c. of starch solution, 1 c.c. of water, and 5 c.c. of saliva solution are placed in one test tube.

5 c.c. of starch solution, 1 c.c. of dilute hydrochloric acid (·1 N or 0·4 per cent.) and 5 c.c. of saliva solution are placed in a second test tube.

5 c.c. of starch solution, 1 c.c. of dilute acetic acid (0·5 per cent.) and 5 c.c. of saliva solution are placed in a third test tube.

5 c.c. of starch solution, 1 c.c. of dilute alkali (·1 N or 0·4 per cent.), and 5 c.c. of saliva solution are placed in a fourth test tube.

5 c.c. of starch solution, 1 c.c. of sodium chloride solution (1 per cent.), and 5 c.c. of saliva solution are placed in a fifth test tube.

The five tubes are placed in the water-bath at 40° and at intervals drops from each are tested with iodine solution.

[1] In experiments with enzymes a control experiment is carried out with boiled enzyme solution instead of water.   The enzyme solution usually contains other substances besides the enzyme and the same amount of these is added to the substrate in each experiment.   It is extremely important to carry out such a control experiment, especially in cases where a rough measurement has to be made to demonstrate enzyme action, e.g. in the cases of lactase and maltase.

Hydrochloric acid completely stops the action of diastase; acetic acid hinders the action, i.e. the conversion of starch into achroodextrin and maltose takes longer; alkali may hasten, but, if strong, will stop the action. A small concentration of sodium chloride hastens the action; sodium chloride of a concentration of 5 per cent. will hinder the action.

Comparison of the Activities of Diastase Solutions.

In the case of diastase, the activity is measured by noticing the time to change starch to dextrin and maltose by disappearance of the reaction with iodine, or by determining the amount of maltose produced in a given time. The former method is more rapid, but the latter gives better results.

A. *The Achromic Method.*

The time taken to effect the change of 1 per cent. starch solution into achroodextrin is measured. The point at which no colour is given by iodine solution, i.e., when the last traces of erythrodextrin have been converted into achroodextrin, is known as the achromic point. The time taken to reach this point is termed the "chromic period." The diastatic power D is the number of c.c. of starch solution which can be converted by 1 c.c. of enzyme solution in 5 minutes, or

$$D = \frac{n}{v} \times \frac{5}{t}$$

where n = number of c.c. starch solution taken, v = volume of enzyme solution (dilution must be known), t = time, 5 = 5 minutes.

The time taken to reach the achromic point must be between 4 and 10 minutes.

The method is carried out as follows:—

5 c.c. of 1 per cent. soluble starch solution, 2 c.c. of 0·5 per cent. sodium chloride solution, and 2 c.c. of a solution of reaction $P_H = 6·7$ [1] are placed in a test tube and warmed to 40° in a water-bath. A series of drops of iodine solution, either on a plate, or in a series of test tubes containing 5 c.c. distilled water, are prepared. 1, or 2, or 3 c.c. of the diastase solution are added to the substrate solution and well mixed, and the time is noted. At intervals of ½ or 1 minute, a drop is removed and tested against a drop of iodine solution. The time is noted when no blue colour is given, the achromic point.

[1] 250 c.c. 0·2M KH_3PO_4 + 105 c.c. 0·2N NaOH made up to 1000 c.c.

If the time is less than 4 minutes, the experiment must be repeated with less enzyme solution, or diluted enzyme solution.

B. *Wohlgemuth's Method.*

This method is carried out with varying quantities of enzyme solution, the disappearance of starch, as shown by the iodine reaction, being taken account of.

5 c.c. of 1 per cent. starch solution are placed in each of a series of 10 tubes and cooled to 0°, whilst an increasing quantity of enzyme solution from 0·1 c.c. to 1 c.c. is added to the series of tubes. They are transferred to a bath at 40° for 30 or 60 minutes and after this time again cooled to 0° to stop the action. Each tube is filled with water and one drop of ·1 N iodine solution is added to each. The colours are blue, blue-violet, reddish, and yellow. The limit is taken as that tube which still shows a violet colour. The activity of the solution is then calculated on the basis of the power of 1 c.c. enzyme solution. Thus, if the tube in the series contain 0·3 c.c. enzyme solution, then the diastatic power at 40° in 30 minutes or $D\dfrac{40°}{30'}$ is $\dfrac{5}{0.3} = 16\cdot6$.

II. GASTRIC JUICE.

The first hydrolysis of proteins occurs in the stomach by the enzyme, pepsin. Pepsin is secreted by certain cells of the gastric mucous membrane. It acts only in the presence of hydrochloric acid, which is secreted by other cells. In disease, lactic acid is sometimes found in the contents of the stomach. Besides pepsin, the enzyme, rennin, is present in the cells of the mucous membrane, but it is very probable that pepsin and rennin are identical. Rennin acts upon caseinogen, the protein of milk, converting it into casein (see under milk, p. 430).

Pepsin.

A solution of pepsin is readily prepared by treating the mucous membrane of the stomach with glycerol for 12-24 hours and straining the solution through muslin. Before use, the glycerol extract is diluted with 2 or 3 volumes of water.

Dry preparations of pepsin in the form of powder, or scales, are obtained by precipitating aqueous extracts with alcohol, or evaporating them to dryness at a temperature below 40°. They generally dissolve slowly in water, or ·1 N hydrochloric acid. A 1 per cent. solution is convenient for the demonstration of pepsin.

As substrate for detecting the presence of pepsin, threads of fibrin, or pieces of coagulated white of egg, are generally used.

* The action of pepsin is demonstrated with five test tubes containing the following mixtures :—

(1) 5 c.c. of water + 1 c.c. of pepsin solution + a piece of fibrin.

(2) 5 c.c. of ·1N HCl + 1 c.c. of water + a piece of fibrin.

(3) 5 c.c. of ·1N HCl + 1 c.c. of pepsin solution + a piece of fibrin.

(4) 5 c.c. of ·1N HCl + 1 c.c. of boiled pepsin solution + a piece of fibrin.

(5) 5 c.c. of ·1N Na_2CO_3 + 1 c.c. of pepsin solution + a piece of fibrin.

The test tubes are placed in a bath at 40°.

Pepsin will only act in the presence of acid ; consequently digestion, or solution, of the fibrin will only take place in the third test tube, where, in about half an hour, the fibrin will have disappeared. In order to show that it is not the acid which has this effect, the second tube, containing no pepsin, but only acid, was used as a control. In this tube the fibrin will have become swollen, but not dissolved. The first, fourth, and fifth tubes will be seen to be unaltered. They both contained pepsin and they show that pepsin will not act in a neutral, or alkaline, medium. Hence pepsin only acts in the presence of acid.

* *Action of Alkali on Pepsin.*

Pepsin is destroyed by the action of dilute alkaline solutions, such as are found in the intestine, where the action of pepsin ceases for this reason ; thus 2 c.c. of dilute sodium carbonate solution are added to 5 c.c. of pepsin solution and it is put in a water-bath at 40° for at least half an hour. It is neutralised with 0·4 per cent. hydrochloric acid, an equal volume of 0·4 per cent. hydrochloric acid and a piece of fibrin are added and it is again kept at 40°. Digestion will not occur.

* *The Products of the Action of Pepsin.*

Proteins are hydrolysed by pepsin and converted into metaproteins, proteoses, and peptones. In a very prolonged digestion amino acids may be formed in small quantities : they are most probably formed by the action of other proteoclastic enzymes—the autolytic enzymes— which have been extracted from the cells of the mucous membrane together with pepsin.

* Several grams (2-5) of fibrin, or egg-white, are placed in ·1N hydrochloric acid solution and 5-10 c.c. of pepsin solution are added. The fibrin dissolves in the course of half to one hour.

The presence of

(1) Metaprotein is shown by neutralising and filtering.

(2) Proteoses by boiling and acidifying the filtrate and testing a portion with concentrated nitric acid. The precipitate, which is formed, dissolves on heating and reappears on cooling. They are removed by saturating the solution with ammonium sulphate.

(3) Peptone by testing the filtrate from the ammonium sulphate precipitate by the biuret reaction in the presence of excess of caustic soda (p. 380).

Comparison of the Activities of Pepsin Solutions.

Numerous methods have been devised for comparing the digestive action of pepsin solutions. The simplest and most convenient is by determination of the rate of solution of an insoluble protein, such as fibrin, or coagulated egg-white. The time taken to dissolve equal quantities is noted. The most convenient method is that of Grützner.

(A) *Grützner's Method.*

Fibrin, stained with carmine, is the substrate used; a definite quantity is added to the enzyme solution and according to the rate of digestion of the fibrin more or less of the dye-stuff passes into solution. Comparison is made by observing the depth of the colour.

Roaf has suggested the use of fibrin stained with congo red instead of carmine. This possesses the advantage that the stained fibrin can be used in both acid and alkaline media; carmine-stained fibrin can only be used in acid media, as carmine is dissolved out of the fibrin by alkalies.

The fibrin is prepared in the following way :—

Fresh fibrin is minced, washed till free from blood, and placed for 24 hours in a 0·5 per cent. solution of congo red in the proportions of 50 gm. of fibrin to 100 c.c. of congo-red solution. The mass is poured into a large volume of water heated to 80° to fix the dye and kept at this temperature for about 5 minutes. The fibrin is then placed in a cloth and washed in running water; the excess of water is squeezed out and the fibrin is preserved in a mixture of equal parts of glycerol and water, a little toluene being added as a preservative.

A known quantity of 0·5 gm. of congo-red fibrin, 5 c.c. of 0·4 per cent. hydrochloric acid, and 5 c.c. of the pepsin solution are placed in a test tube and put in a water-bath at 40° for half an hour. After this time, sufficient solid anhydrous sodium carbonate is added to change the blue colour of the congo red to red. This also stops the action of the

enzyme. A measured volume is removed; in order to compare two pepsin digests water is added from a burette to the deeper one till the tints of the two solutions are the same. The amount of water added is noted. The strengths of the enzymes are to one another as the amount of dilution: thus, if an equal volume of water be added the strengths are as 2 : 1.

(B) *Mett's Method.*

Numerous results, especially those in Pavloff's laboratory, upon digestion by enzymes have been obtained by this method. It consists in directly measuring the amount of protein digested in a given time, the protein being contained in narrow tubes open at both ends and known as Mett's tubes.

Mett's tubes consist of a small length of coagulated egg-white, or serum, in a narrow glass tube of 2 mm. bore, and are made by drawing up egg-white into the glass tubing (no air bubbles must be present in the egg-white) and placing it in nearly boiling water for 2-3 minutes. This tubing is then cut up into lengths of about 1 cm. The coagulated egg-albumin must form a continuous layer free from air spaces. Two small pieces of tube are placed in the enzyme solution and after a definite lapse of time the pieces are laid upon a mm. scale and the amount dissolved from each end measured. The mean of these readings is taken.

5 c.c. of 0·4 per cent. hydrochloric acid, 5 c.c. of pepsin solution, and 1 or 2 Mett's tubes are placed in a small conical flask. The flask is stoppered and kept at 37° for 8-10 hours. It is then removed and the amount of protein digested from each end of the Mett's tubes is measured. The mean is taken. In this experiment where the action is for a long period of time, according to the Schütz law, the squares of the lengths digested represent the activity of the enzyme more accurately than the direct ratio.

(C) *Fuld's Method.*

By making use of edestin as substrate and its precipitability by salts from its solution in hydrochloric acid, Fuld has devised a very simple method for measuring the activity of pepsin solutions. Varying amounts of the enzyme are added to definite volumes of the edestin solution in a series of tubes and after a prescribed lapse of time sodium chloride is added; the first tube in each series, in which a precipitate of edestin is no longer formed, is noted, i.e. the tube containing least enzyme. Thus :—

5 c.c. of the 0·5 per cent. edestin solution in 0·4 per cent. hydrochloric acid are measured out with a pipette into each of a series of five test tubes. To these tubes is added in order, 0·2 c.c., 0·4 c.c., 0·6 c.c., 0·8 c.c., 1·0 c.c. of the pepsin solution A from a burette (generally 2 drops = 0·1 c.c.). The same operations are performed with pepsin solution B.

The tubes are kept at the ordinary temperature for half an hour, or longer, but the same time for each series; then to each tube is added 1 c.c. of saturated sodium chloride solution. The first tube in which a precipitate of edestin is no longer produced is noted.

* (D) *Hata's Method.*

Hata, in 1909, showed that a suspension of coagulated egg-white was a very delicate substrate for estimating the activity of pepsin solutions. Under the influence of the enzyme the cloudy solution becomes quite clear.

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The substrate is prepared by rubbing up egg-white in a basin until it is of a uniform consistency. It is then slowly mixed and rubbed up with water until it has been diluted five times. The solution is strained through muslin and heated in a water-bath at 60° for 20 minutes, or until it becomes distinctly opalescent, after which it is once more strained through muslin. A homogeneous suspension is thus obtained. Before use, it is diluted with 9 volumes of water.

5 c.c. of the above substrate are measured out into each of a series of five test tubes. To each is added 5 c.c. of 0·4 per cent. hydrochloric acid solution and then in order, 0·2, 0·4, 0·6, 0·8, and 1 c.c. of pepsin solution. The tubes are placed in a water-bath at 40° for 15 or 30 minutes. It is noted in which tube in the series the smallest amount of enzyme first produces complete clarification.

(E) *Gross' Method.*

Gross has suggested a solution of caseinogen instead of edestin for estimating peptic activity. It is prepared by dissolving 1 gm. of pure caseinogen in 16 c.c. of 25 per cent. hydrochloric acid of sp. gr. 1·124 in a 1000 c.c. flask in a water-bath and diluting to 1000 c.c. It is precipitated by a 20 per cent. solution of sodium acetate.

A series of tubes are filled with varying quantities of the pepsin solution from ·1-1 c.c. To each, 10 c.c. of the caseinogen solution warmed to 40° are added. The series is placed in a bath at 38-40° for 15 minutes. A few drops of the sodium acetate solution are added to each tube; undigested caseinogen is precipitated. The smallest quantity required to digest the 10 c.c. is the value noted.

If 1 c.c. of pepsin solution be the basis of the calculation and ·025 c.c. were sufficient, the pepsin solution corresponds to $\cdot\frac{1}{025}$ or 40 units.

(F) *Calcified Milk.*

The use of calcified milk for comparing proteoclastic activities is probably the most convenient method. The time is taken at which a clot is produced by a known volume of enzyme solution. Clotting of milk is a property of most proteolytic enzymes, and is not confined to the action of rennin.

Calcified milk is prepared by adding 10 c.c. of N calcium chloride solution (5·55 per cent.) to 50 c.c. of fresh milk.

A clotting time of 75-105 seconds is generally adopted as a standard. Several trials to procure an enzyme solution acting in this time are generally needed.

A series of 4 or 5 tubes containing 5 c.c. of calcified milk is prepared. They are placed in a bath at 38°. In all experiments the final volume must be made to 6 c.c.

The first trial is made with 1 c.c. pepsin solution, which is added and quickly mixed with the substrate by shaking. Clotting, or precipitation, is noticed and the time is taken.

If too rapid, less enzyme solution, or diluted enzyme, is taken and if too slow, more enzyme solution must be used until the suggested clotting time is arrived at.

Cole takes the time to clot 5 c.c. milk in 100 seconds as unit.

The time of clotting is inversely proportional to the amount of enzyme, e.g. 1 c.c. of a pepsin solution, diluted 1 in 4 clots in 95 seconds. The enzyme solution then contains $4 \times \frac{100}{95}$ units of pepsin.

THE ACIDS IN THE GASTRIC CONTENTS.

Normally hydrochloric acid to the extent of about 0·2-0·4 per cent. is secreted by the gastric mucous membrane, but in disease it may be absent and lactic acid may be found.

Detection of Mineral Acid.

The presence of an acid in a solution is shown by the colour change it produces in an organic dye-stuff, or indicator. In pure aqueous solutions, mineral acids give a distinct colour change, organic acids give a less distinct colour change. The colour change is masked in the presence of proteoses and peptones which are present in the gastric contents, owing to the combination of the acid with the protein in the form of a salt, so that it is difficult to decide whether hydrochloric acid is present, or absent.

A *marked* colour change in a series of indicators, such as methyl violet, congo-red, methyl orange will show the presence of hydrochloric acid, but a slight colour change is not decisive.

The presence of free hydrochloric acid is shown only by means of Gunzberg's reagent.[1]

The test with Gunzberg's reagent is carried out thus :—

About 5 drops of the stomach contents are placed in a small basin, 2-3 drops of *freshly prepared* Gunzberg's reagent are added, and they are evaporated very carefully over a small flame, oscillating the basin and blowing upon the mixture. Charring must be prevented. A brilliant red tinge is formed on the edge of the dried residue in presence of free hydrochloric acid.

Estimation of the Acids.

The estimation of the amount of free hydrochloric acid, combined hydrochloric acid, and organic acid in gastric contents by Töpfer's method of titrating with (1) phenolphthalein, (2) dimethylaminoazobenzene to an orange-yellow, not yellow tint, (3) alizarin red, is not satisfactory. It was supposed to give the following information :—

(1) total acids, i.e. free mineral acid + combined mineral acid + organic acid.

(2) free mineral acid.

(3) free mineral acid + organic acid.

(1) minus (3) combined mineral acid.

(3) minus (2) organic acid.

[1] Gunzberg's reagent—2 gm. phloroglucinol, 1 gm. vanillin, 30 gm. absolute alcohol.

The data are not absolutely correct, but they serve for comparative determinations.

It is more exact to determine (1) total acids, (2) total chlorides, (3) free hydrochloric acid.

Total Acids.

Total acids are determined by titrating 10 c.c. of filtered stomach contents with 0·1 N sodium hydroxide using 4-5 drops of phenolphthalein as indicator.

The result is expressed in gm. HCl per 100 c.c.

$$(1 \text{ c.c. } \cdot 1 N = 0 \cdot 00363 \text{ gm. HCl}).$$

Total Chlorides.

Total chlorides are determined by titrating 10 c.c. of filtered stomach contents by Volhard's method (see urine, p. 521).

It is considered preferable to add 1 c.c. of saturated sodium carbonate solution to 10 c.c. of stomach contents in a crucible or basin, evaporate to dryness, and incinerate at a low red heat. The ash is dissolved in dilute nitric acid, put into a 100 c.c. measuring cylinder, treated with 10 c.c., or more, of silver nitrate, made up to volume, and treated according to Volhard's method.

Mineral Chlorides.

10 c.c. of stomach content are evaporated and incinerated. Free and combined hydrochloric acid are volatilised. The remaining chlorides are estimated by Volhard's method.

Note.—These estimations are based upon the belief that sodium chloride is not volatilised on heating. Sodium chloride is, however, volatilised on heating, especially at any high temperature.

Active Hydrochloric Acid.

The difference between total chlorides and mineral chlorides is active hydrochloric acid.

Free Hydrochloric Acid.

Free hydrochloric acid may be determined by the use of Gunzberg's reagent. 10 c.c. of filtered stomach contents are treated with 1 c.c. of ·1 N NaOH. One drop is removed and tested with 1 drop of Gunzberg's reagent, as described above.

If the test is positive, it is repeated after adding another c.c. of ·1 N NaOH. The test is repeated till the test is negative. Say 3 c.c. altogether have been added.

·1 N HCl, 0·1 c.c. or 0·2 c.c. at a time are now added, and a drop is

tested with a drop of Gunzberg's reagent, till the test becomes positive. Say 0·6 c.c. have been used.

The result is thus

10 c.c. stomach contents + 3 c.c. ·1N NaOH + 0·6 c.c. ·1N HCl.

Free HCl = 3 − 0·6 = 2·4 c.c. ·1N HCl per 10 c.c. stomach contents.

Combined Hydrochloric Acid.

The difference between active hydrochloric acid and free hydrochloric acid is combined hydrochloric acid.

Organic Acids.

The difference between total acidity and active hydrochloric acid is acid due to organic acids.

III. PANCREATIC JUICE.

From the stomach the food passes into the intestine. The acid contents of the stomach, when they pass into the duodenum and come in contact with its mucous membrane, induce the secretion of secretin. Bile, also, as it enters the duodenum, induces the production of secretin. Its action is due to presence of cholic acid in combination as bile acids, and to the presence of mucin (J. Mellanby).

Secretin passes into the blood and is carried to the pancreas, where it excites a flow of pancreatic juice. This juice has very little action upon proteins, but it contains lipase and diastase which hydrolyse fats and starch respectively. As soon as the pancreatic juice, which contains trypsinogen, comes into the intestine, it becomes activated by the enzyme, enterokinase, and converted into the powerful proteoclastic enzyme, trypsin. Enterokinase is secreted by the glands of the duodenum. Trypsin acts upon unchanged proteins, proteoses, etc., from the stomach and converts them almost entirely into amino acids. A complex polypeptide is also formed which is not acted upon by trypsin.

Preparation of Pancreatic Juice.

The mucous membrane of the small intestine is ground up with sand and boiled with dilute hydrochloric acid. The boiling solution is neutralised with dilute alkali. Coagulable proteins are thus precipitated and filtered off. The solution contains *secretin*. Secretin is not an enzyme as it can be boiled, but belongs to the class of substances termed *hormones* by Professor Starling.

A cannula is placed in the pancreatic duct and the solution of secretin is slowly injected into the jugular vein. Pancreatic juice flows from the cannula after each injection and is collected in a clean vessel. It is mixed with an equal volume of 2 per cent. sodium fluoride to preserve it.

Activation by Enterokinase.

Conversion of Trypsinogen into Trypsin.

A solution of enterokinase is prepared by making an aqueous extract of the mucous membrane of the upper part of the small intestine.

(*a*) As substrate a capillary tube (Mett's tube, cf. p. 449) of 1-2 mm. bore about 2 cm. long and filled with coloured gelatin is generally used. These are prepared by drawing up hot 10-20 per cent. gelatin solution stained with methylene blue, or gentian violet, into the tube, placing the tube horizontally and allowing the gelatin to set. The tube is cut into pieces 1-2 cm. long. They can only be used for experiments at room temperature; at 40° the gelatin melts and flows out of the tube.

Two of these tubes are placed in each of three small conical flasks together with 5 c.c. of ·5 per cent. sodium carbonate solution.

In the first is placed 1-2 c.c. of pancreatic juice, or trypsinogen, solution.

In the second is placed 1-2 c.c. of pancreatic juice, or trypsinogen, solution + a few drops of enterokinase solution.

In the third is placed 1-2 c.c. of boiled pancreatic juice, or trypsinogen, solution and a few drops of enterokinase solution.

The flasks are kept at room temperature for 8-10 hours.

No solution, or digestion, of the gelatin occurs in No. 1, or No. 3, which contained the trypsinogen, or the boiled trypsinogen, but in No. 2 the gelatin will have been dissolved at both ends of the capillary tube.

(*b*) H. Bierry and V. Henri have shown that milk is a very sensitive substrate for observing the activation of pancreatic juice by enterokinase. The milk is centrifugalised and filtered from fatty particles through wet paper and is sterilised by heating.

In four clean test tubes are placed :—

(1) 5 c.c. of milk + 5 drops of pancreatic juice.

(2) 5 c.c. of milk + 5 drops of pancreatic juice + 2 drops of intestinal extract.

(3) 5 c.c. of milk + 5 drops of boiled pancreatic juice + 2 drops of intestinal extract.

(4) 5 c.c. of milk + 2 drops of intestinal extract.

They are put in a water-bath at 40° for 10-15 minutes. No change will be found to have occurred in tubes No. 1, No. 3, and No. 4, whereas in No. 2 there is an immediate clarification of the milk, which becomes transparent after the lapse of the above time.

Trypsinogen.

Trypsinogen may be prepared from the pancreas by the method described by Mellanby and Woolley :—

The pancreas is removed without contact with the intestines. It is finely minced and treated with twice its weight of ·5 per cent.

hydrochloric acid at room temperature for 12 hours. The solution is strained through muslin and neutralised with sodium carbonate. The precipitate is filtered off and the solution is kept under toluene.

Trypsin.

Trypsin is the activated proteoclastic enzyme of the pancreas. It may be prepared by extracting the minced gland with glycerin for 12-24 hours and straining through muslin. The solution is diluted with 2-3 volumes of water before use.

An active solution of trypsin may also be prepared by treating the minced pancreas with three times its weight of distilled water and an equal weight of alcohol for 3 days at room temperature with occasional shaking. The solution is strained through muslin and filtered. To the filtrate 1 c.c. of concentrated hydrochloric acid per 1000 c.c. is added. A precipitate which forms is allowed to settle and is filtered off.

In preparing trypsin from the pancreas it is advisable to add a small amount of the mucous membrane of the intestine, so as to activate the enzyme, in case this is not done by contact with the intestine on removing the pancreas.

Dry preparations of trypsin can be obtained by mincing the pancreas and drying, or by precipitating with alcohol, or evaporating the extracts.

Numerous preparations of trypsin can be obtained commercially, e.g. Benger's liquor pancreaticus, holadin of Messrs. Fairchild Bros. & Foster. This latter preparation also contains lipase and diastase.

Solution of fibrin, or coagulated egg-white, by the enzyme, as in the case of pepsin, is the simplest means of investigating the presence and action of trypsin.

Four test tubes are filled with the following mixtures :—

(1) 5 c.c. of trypsin + 5 c.c. of ·5 per cent. Na_2CO_3 + a piece of fibrin.

(2) 5 c.c. of trypsin + 5 c.c. of water + a piece of fibrin.

(3) 5 c.c. of trypsin + 5 c.c. of ·1 N HCl + a piece of fibrin.

(4) 5 c.c. of boiled trypsin + 5 c.c. of ·5 per cent. Na_2CO_3 + a piece of fibrin.

The four tubes are placed in a water-bath at 40°. Only in the first tube will any change be seen. In the tube containing hydrochloric acid the fibrin swells without dissolving. In the aqueous solution there is no visible change, nor in the tube containing boiled enzyme. Trypsin thus acts only in faintly alkaline solution of 0·2-0·5 per cent. concentration.

The Products of the Action of Trypsin.

Proteins are hydrolysed by trypsin and converted into amino acids (and a polypeptide).

The presence of the amino acids can be shown in a digest of protein, prepared by treating about 100 gm. of caseinogen, or other

protein, dissolved in 2000 c.c. of ·1N ammonia, or sodium carbonate, at 37° for several days with about 1 gm. of dried pancreas preparation in the presence of toluene or chloroform.

(1) The solution will most probably contain a white precipitate, which consists mainly of tyrosine. This is proved by filtering it off, washing and dissolving it in dilute acetic acid and testing with Millon's reagent (p. 211).

(2) The filtrate from the tyrosine can be shown to contain trypto-phan by acidifying a portion of about 5 c.c. with acetic acid and add-ing bromine water, drop by drop, as described on p. 354.

(3) On evaporating the filtered solution on a water-bath to a small volume and allowing to stand for about 24 hours, a crystalline crust forms. This con-sists mainly of tyrosine as can be shown by microscopic examination, especially after solution in a drop of ammonia. The crystals will also give Millon's reaction.

(4) On further evaporation of the filtrate, leucine and glutamic acid sep-arate out on standing. Microscopic examination will show that the crystals consist mainly of rounded cones with a radiating striation (leucine). If free from tyrosine, they will not give Millon's reaction. They dissolve in hot water and form copper salts, as can be shown by adding a very little caustic soda and a few drops of copper sulphate. The precipitate of cupric hydroxide dissolves, on warming, giving a blue solution.

Pancreatic Diastase.

The diastase of the pancreas does not hydrolyse properly except in the presence of salts; ·3 gm. of sodium chloride and 7 c.c. of ·2N disodium phosphate should be added per 100 c.c. of reaction mixture.[1]

A solution containing diastase can be prepared from the pancreas of animals by allowing a fresh pancreas (free from fat), which has been finely minced, to stand with twice its weight of glycerol, for 12 - 24 hours and straining through muslin. Before use the solution may be diluted with 1-2 volumes of water, or preferably 1-2 drops of the concentrated extract may be used. Diastase is present in pancreatic juice and may be detected in ·1 to 1 c.c.

Pancreatic Lipase.

(a) The lipase in pancreatic juice may be demonstrated directly by adding a few drops, or 1 c.c., to neutral olive oil (about 5 c.c.) contain-ing a drop of phenolphthalein. The mixture is coloured red by running in ·1N alkali from a burette. On keeping warm at 40° and occasionally shaking, decolorisation takes place. More alkali is run in, drop by drop, until the red colour again appears. It will disappear again. This can be repeated several times.

[1] Sherman, Kendall and Clark, J. Amer. Chem. Soc., 1910, **32**, 1073.

(*b*) Lipase from pancreas.

The pancreas is freed from fat, weighed, finely minced and ground up with sand. It is then extracted for 24 hours with a mixture consisting of 90 parts of pure glycerol and 10 parts of 1 per cent. sodium carbonate solution, 10 c.c. of this mixture being used for every gram of pancreas. The fluid is strained through muslin and is kept at 0°. The lipase is destroyed as soon as the fluid becomes acid; this happens generally in about three days.

An active extract may also be prepared by treating the fresh and finely minced pancreas with twice its weight of ·5 per cent. sodium carbonate solution for 12 hours and straining through muslin.

Effect of Bile Salts on Pancreatic Lipase.

* Bile salts increase the rate of hydrolysis of fats by lipase and act as a co-enzyme. This can be demonstrated by the following three experiments :—

(1) 5 c.c. neutral oil + 5 c.c. pancreas extract + 1 c.c. of water.

(2) 5 c.c.   ,,   ,, + 5 c.c.   ,,   ,,   + 1 c.c. of 1 per cent. bile salt solution.

(3) 5 c.c.   ,,   ,, + 5 c.c. boiled ,,   ,,   + 1 c.c. of 1 per cent. bile salt solution.

These three mixtures are kept at 40° for half an hour and titrated with ·1 N alkali, using phenolphthalein as indicator. No. 3 requires least alkali, No. 2 requires most. More hydrolysis therefore occurs in the presence of bile salts.

Comparison of the Activities of Trypsin Solutions.

The activity of trypsin solutions is measured by solution of an insoluble substrate, by the clotting of milk, or by determination of the rate of production of amino acids.

(*a*) *Mett's Method.*

The activity of a trypsin solution can be measured by Mett's method in the same way as described for pepsin. The tubes may contain either egg-white, or gelatin (p. 454). 5 c.c. of ·4 per cent. sodium carbonate solution and 5 c.c. of the trypsin are placed in a small conical flask together with 1 or 2 Mett's tubes. 1 c.c. of toluene, or chloroform, is also put in the flask as antiseptic. After periods of 2, 4, 8-24 hours the lengths digested from each end are measured.

The squares of the lengths digested more nearly represent the activity than the actual lengths which are measured.

(*b*) *Calcified Milk.*

Calcified milk, prepared as described on p. 450 may be used for comparing tryptic activities.

The procedure is the same as with pepsin, p. 450 and a definite unit can be prescribed.

*   (c) *Sörensen's Method.*

The simplest method is that of Sörensen.   In this method, the rate of formation of the products of the action of the enzyme, namely, the amino acids, is measured.   The amino acids contain both a carboxyl group and an amino group and consequently their reaction is neutral. By combining the amino group with formaldehyde, its basic character is destroyed and the carboxyl group is free to exert its acid character. The reaction which takes place is :—

$$R.CH.NH_2 + OHCH \quad = \quad R.CH.N:CH_2 + H_2O$$
$$\overset{\displaystyle |}{COOH} \qquad\qquad \overset{\displaystyle |}{COOH.}$$

Samples of a trypsin digest of caseinogen, gelatin, etc., are treated at intervals with neutral formaldehyde.   They show a gradual increase in acidity as the action of the enzyme proceeds ; the rate of the increase depends on the strength of the enzyme.

*   60 c.c. of formalin are diluted with two volumes of water and neutralised by running in ·1 N alkali from a burette until the colour is just red to phenolphthalein, which is added as indicator.

100 c.c. of a 4 per cent. caseinogen solution in 0·4 per cent. sodium carbonate solution are measured out into a small flask, warmed to 40° and then 5 c.c., or more, of the trypsin solution are added ; the mixture is kept at 40°.   Immediately after the addition, a sample of 25 c.c. is removed with a pipette and 30 c.c. of the previously neutralised formaldehyde solution are added.   At intervals of half an hour, one hour, one hour and a half, two hours, further samples of 25 c.c. are removed and to them are added 30 c.c. of the formaldehyde solution and a few drops of phenolphthalein.   Each sample, as it is obtained, is titrated with the ·1 N alkali in the burette until the solution has a distinctly red colour.   The amount of alkali used for each sample is noted.

IV. INTESTINAL JUICE.

The cells of the mucous membrane of the small intestine produce a secretion—the succus entericus—which contains erepsin, a peculiar proteoclastic enzyme discovered by Cohnheim ; it acts only upon proteoses and peptones, converting them into amino acids.   This juice also contains invertase and lactase.   These enzymes act not only in the secreted juice, but also inside the cells of the mucous membrane.   Any unhydrolysed protein, or polysaccharide, is hydrolysed into its constituents.

Erepsin.

A solution of erepsin is prepared by grinding the mucous membrane with sand and treating with water, to which 1 per cent. of toluene has been added, for 12-24 hours. The solution is strained from sand and connective tissue through muslin. Erepsin acts upon proteoses and peptones forming amino_acids; a 2 per cent. solution of Witte's peptone is therefore used as substrate.

Two portions of Witte's peptone solution (500 c.c.) are placed in bottles; to one portion 100 c.c. of erepsin solution are added; to the other portion 100 c.c. of boiled erepsin solution. To both are added 6 c.c. of toluene and they are kept at 37° for 1-3 days.

A portion of each is examined for proteoses and peptones by the biuret reaction by adding exactly the same amount of caustic soda solution (5 c.c.) and exactly the same amount of 1 per cent. copper sulphate solution.

The solution which contained boiled enzyme will show the biuret reaction (the substrate is unchanged).

The solution which contained enzyme will either not show the biuret reaction, or it will be fainter than in the other solution (the substrate has been hydrolysed completely, or not quite completely).

Invertase.

The succus entericus, or an extract of the cells of the mucous membrane of the small intestine, prepared by grinding the material with sand, allowing it to stand with water for 12 hours in the presence of toluene and straining through muslin, is divided into two equal portions. One portion is boiled and cooled. To each portion is added an equal volume of 1 per cent. cane sugar solution. 1 per cent. of toluene is added to each and the two mixtures are kept at 37° for 12-24 hours. Each solution is tested with Fehling's solution. Reduction only occurs where the unboiled extract is present showing the presence of invertase.

Lactase.

The mucous membrane is scraped off, ground up with sand to break the cells, and kept in water with 1 per cent. of toluene for 12-24 hours. The solution is strained through muslin and divided into two nearly equal parts, say of 105 and 110 c.c. The larger part is boiled and cooled.

100 c.c. of unboiled solution are added to 100 c.c. of 5 per cent. lactose solution in a small flask (1). 100 c.c. of boiled solution are

added to 100 c.c. of 5 per cent. lactose solution in a small flask (2). 1 c.c. of toluene is added to each; the two flasks are corked and kept at 37° for 1-4 days.

Flask (1) will show a reduction when tested with Barfoed's reagent.

Flask (2) will not show a reduction; if there is a slight reduction, this will be due to the presence of glucose in the extract.

V. THE LIVER.

The liver is concerned most intimately with the products formed during digestion in the intestine, especially the monosaccharides and the fats, and with the regulation of their amount in the blood.

Monosaccharides, chiefly glucose, are converted into glycogen and retained as reserve food-stuff to be broken down again when the amount of glucose in the blood sinks below its normal limit. 1 to 2 per cent. of glycogen is present in the livers of well-nourished animals, but generally it varies from ·1-·5 per cent. According to Külz, the greatest amount is present in the liver 14-16 hours after a meal.

The fats undergo changes in the liver cells—the saturated become unsaturated, and they are oxidised to simpler fatty acids and hydroxy acids which circulate in the blood.

The amino acids undergo deaminisation in the liver as well as in the other tissues. Liver cells contain arginase, which hydrolyses arginine to urea and ornithine. The liver is further concerned in the formation of urea from ammonia and carbon dioxide; in birds with that of uric acid as well.

The liver breaks down the hæmoglobin of the blood and excretes the products, bilirubin and biliverdin, into the intestine through the bile duct. Bile salts are also passed into the bile.

VI. AUTOLYSIS.

Proteoclastic and other enzymes are present in all tissues; they are concerned in the breaking down of the constituents of the tissue and in their synthesis.

They are the cause of the auto- or self-digestion of the tissues after death; in starvation, food is supplied by the breaking down of some organs at the expense of other organs by the autolytic enzymes.

VII. PUTREFACTION.

In the large intestine food remains are acted upon by bacteria. Amino acids are formed from proteins and they are further broken

down into amines, carbon dioxide, indole, scatole, hydrogen sulphide, fatty acids. Unabsorbed carbohydrates and fats are also hydrolysed and decomposed.

THE CONSTITUENTS OF BILE.

The constituents of the bile are :—

(1) The colouring matters, bilirubin and biliverdin, the latter formed by the oxidation of the former.

(2) The bile salts, the sodium salts of glycocholic and taurocholic acids.

(3) A small quantity of mucin, or nucleoprotein (the more recent work insists on the presence of the latter, but both are probably present).

(4) Cholesterol, which gives rise to gall-stones in certain conditions.

Examination of Ox, or Sheep, Bile.

* (1) It has a faintly alkaline reaction to litmus ; the bitter taste and peculiar odour should be noticed.

* (2) It does not coagulate on heating.

* (3) On acidifying a small quantity with acetic acid, a precipitate is formed which is insoluble in excess of acetic acid. As above stated, this precipitate was considered to be mucin owing to its insolubility in excess of acid, nucleoprotein being soluble ; in the presence of bile salts the precipitate of nucleoprotein is insoluble.

* (3a) No pigment is extracted on shaking up a little bile with ether. If a few drops of dilute hydrochloric acid be added, both nucleoprotein and pigment are liberated as free acids from their sodium compounds. The nucleoprotein is precipitated, but the pigment passes into solution on shaking with the ether.

* (4) **Gmelin's Test for Bile Pigments.**—A little bile is carefully placed on the surface of some fuming nitric acid in a test tube, either by pouring it carefully down the side of the tube, or by means of a pipette. On shaking the tube very gently, a play of colours will be seen as the bile becomes oxidised. Generally, the colours are yellow, red, violet, blue, green ;

<div align="center">or,</div>

A drop of fuming nitric acid is placed on a thin film of bile in a porcelain basin. Rings of the various colours will be seen ;

<div align="center">or,</div>

A little bile is filtered several times through an ordinary filter paper and a drop of fuming nitric acid is placed on the paper. The colours will be seen.

*   (5) **Huppert's Test for Bile Pigments.**—This test is especially useful for detecting bile pigments in urine.

5 c.c. of bile are diluted with 25-50 c.c. of water and 4 c.c. of sodium phosphate solution and 6 c.c. of calcium chloride solution are added. The precipitate is filtered off. It carries down the pigment mechanically, or may contain an insoluble calcium compound' of bilirubin; it is heated with 5 c.c. of alcohol and a few drops of concentrated hydrochloric acid. A fine green colour is formed. The formation of the green colour may require the addition of an oxidising agent such as a few drops of ferric chloride, or potassium chlorate, solution (Cole).

*   (6) **Pettenkofer's Test for Bile Salts.**—A fragment of cane sugar is dissolved in a little bile which has been diluted 10 times with water; when it has dissolved, about 5 c.c. of concentrated sulphuric acid are run into the bottom of the test tube and shaken gently. A purple colour develops slowly. Furfural is formed by the action of the concentrated sulphuric acid on the sugar; this reacts with the bile acids, giving the purple colour. Excess of sugar must be avoided as it may be charred by the strong acid and spoil the colour. The colour disappears on diluting with water and is only stable in the presence of strong sulphuric acid. If a portion of the purple liquid be diluted with 50 per cent. sulphuric acid and examined in the spectroscope, two absorption bands, the one between C and D, nearer D, and the other in the green, can be observed.

This test is sometimes performed by shaking up the bile with a little sugar solution so as to obtain a froth; on pouring in the concentrated sulphuric acid, the colour appears where it has come in contact with the froth.

*   (7) **Hay's Test for Bile Salts** (Surface Tension Test).—A little bile in a test tube is diluted with water and some flowers of sulphur are sprinkled on the surface. They sink. If the experiment be repeated with pure water, the particles of sulphur will float.

On performing the same test with strong mineral acids, ammonia, brine, ammonium sulphate solution, etc., the sulphur floats. It sinks in alcohol, ether, chloroform, olive-oil, etc., in fact in all liquids with a surface tension less than 60 dynes per sq. cm.

This test depends upon the power of the bile salts to lower the surface tension of water. It is particularly valuable for detecting bile salts in urine where other coloured substances may interfere with Pettenkofer's test. Alcohol, which has a low surface tension, must, if present, be previously removed by evaporation.

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Grunbaum has described a method of estimating bile salts in urine which depends on this property of bile salts. The rate of escape of urine from standard capillary tubes is measured ; the higher the concentration the greater is the rate.

Draughtsmen employ this property of bile salts in making tracings on oiled paper on which the ink collects in drops and does not spread. On treating the paper with ox bile and allowing it to dry, the difficulty is overcome owing to the reduction in surface tension. This experiment with oiled paper treated with bile may be tried with advantage.

In the same way oil will pass through a filter paper moistened with dilute bile solution, whereas it will not pass through a paper moistened with water. This statement may easily be verified.

(8) **Solvent Action of Bile Salts on Fatty Acids containing Oleic Acid.**—If some fatty acids from mutton, or beef, fat be stirred with water, they do not dissolve; on adding a little bile and stirring up again, fatty acids can be detected in the filtrate by evaporating to dryness, heating and noting the characteristic odour. This process no doubt occurs in the digestion and absorption of fats.

(9) **Oliver's Test for Bile Salts.**—This depends upon the power of the bile acids to precipitate peptone in acid solution and is useful for showing the presence of bile salts in the urine, e.g.—

About 20 c.c. of bile are evaporated to complete dryness on the water-bath. The residue is heated with 20 c.c. of alcohol on the water-bath, stirring the mixture thoroughly with a glass rod. A little more alcohol is added and it is filtered. The filtrate is evaporated to dryness on the water-bath and the residue extracted with about 30 c.c. of hot water. A solution of the pigments and the salts of bile, free from proteins, is obtained on filtering.

If a portion of this solution be acidified with glacial acetic acid, the bile acids are not thrown down, but on adding an equal quantity of 1 per cent. Witte's peptone solution a turbidity, or a precipitate, is obtained, insoluble in excess of acid.

As applied to urine, it is only necessary to acidify with acetic acid, filter till quite clear and treat with an equal volume of 1 per cent. Witte's peptone solution.

* (10) **Cholesterol** may be detected as follows (Roaf) :—

10 c.c. of bile are evaporated to dryness on the water-bath. The residue is extracted several times with small quantities of ether, pouring each ether extract into an evaporating basin. The ether is allowed to evaporate and the residue is dissolved in about 2 c.c. of chloroform. It gives Salkowski's and Liebermann's reactions.

GALL STONES.

Calculi of various sizes and shapes and of variable number occur in the gall bladder. Three kinds have been distinguished :—

(1) Pigmented Chalk Stones.

In man, these stones are small; in the ox and pig, stones as large as a walnut have been found. They are heavier than water. They consist almost

entirely of the calcium salt of bilirubin and contain very little, or no, biliverdin. Sometimes black, or greenish-black, metallic-looking stones, which consist of bilifuscin and biliverdin, occur.   Iron and copper are generally present.

(2) Cholesterol Stones.

The shape and size of the cholesterol stones are very variable ; they are generally lighter than water and are composed of concentric layers.   Their surface, if fractured, appears crystalline ; if cut, waxy.   If the fractured surface be rubbed with the nail, it also looks waxy.   By rubbing against one another in the gall bladder they are generally faceted.   They are almost white and usually show pigmented edges (pigmented chalk).

(3) Calcium Carbonate and Phosphate.

These stones are very rare in man.

BLOOD is a fluid which contains proteins, salts, glucose, amino acids and other simple compounds in solution, and red and white blood corpuscles in suspension. The presence of blood platelets in living blood, i.e. in the blood vessels, is denied by some observers, but they are undoubtedly present when the blood is shed, their formation being instantaneous.

Composition of Blood.

Blood plasma consists mainly of water; the solid matter amounts to less than 10 per cent. The proportions of the constituents have been frequently determined and some of the analyses for 1000 c.c. are given in the following tables :—

	Horse.		Man and other Animals.	Chicken.	Frog.
	Hoppe-Seyler.	Hammarsten.			
Water	908·4	917·6			
Solids	91·6	82·4	70 to 97	54	
	1000·0	1000·0			
Fibrin	10·1	6·5			
Globulin . . .	—	38·4			
Albumin . . .	—	24·6			
Total Proteins . .	77·6	69·5	55 to 8⅟	39·5	25·4
Fat	1·2				
Extractives . . .	4·0				
Soluble Salts . . .	6·4	13·3	12·9		
Insoluble Salts . .	1·7				

	Total Protein.	Fibrinogen.	Globulin.	Albumin.	
Man	72·6	4·2	28·3	40·1	
Dog	60·3	6·0	22·6	31·7	by
Sheep	72·9	4·6	30·0	38·3	Lewinsky.
Horse	80·4	4·5	47·9	28·0	
Pig	80·5	6·5	29·8	44·2	

The ratio globulin to albumin in man is usually 1 : 1·15 but 1 : 1·39 to 2·13 has been found. In starvation the globulin increases.

		Man (by Schmidt).	Other Animals (by Bunge and Abderhalden).
K_2O	. . .	0·387 to 0·40	0·225 to 0·270
Na_2O	. . .	4·290	4·251 to 4·442
CaO	. . .	0·155	0·119 to 0·131
MgO	. . .	0·101	0·040 to 0·046
Cl .	. . .	3·565 to 3·659	3·627 to 4·170
P_2O_5	. . .	—	0·052 to 0·085

COAGULATION OF BLOOD.

Blood drawn from a blood vessel clots spontaneously into a solid mass, or clot. On standing, the clot slowly contracts, expressing the almost colourless blood-serum. The clot consists of the insoluble protein, fibrin, which has entangled the blood corpuscles. The fibrin, almost free from corpuscles, can be obtained by whipping the blood whilst it clots and washing the fibrin threads with water to remove the entangled corpuscles; defibrinated blood remains, which contains the corpuscles. Thus

$$
\text{Blood}\begin{cases}\text{Fibrin + Corpuscles} \\ \\ \text{Serum}\end{cases}
\qquad
\begin{matrix}\text{Whipped} \\ \text{Blood}\end{matrix}\begin{cases}\text{Fibrin} \\ \\ \text{Defibrinated Blood} \\ \text{(Serum + Corpuscles)}\end{cases}
$$

This may be readily observed as follows :—

A little freshly drawn blood is collected in two watch glasses. The one is allowed to clot; after a time the clot contracts, expressing the serum. The other is defibrinated by stirring it with a pin, or needle. The fibrin adheres to the needle and defibrinated blood remains. The fibrin can be washed free from corpuscles by water and an almost colourless mass remains.

Factors Concerned in Clotting of Blood.

In the process of clotting the insoluble protein, fibrin, is formed. It arises from fibrinogen, a soluble protein contained in the blood plasma, by the action of fibrin ferment, or thrombin.

Thrombin does not exist normally in the blood, but is formed from precursors after the blood is shed in the presence of calcium salts. The older observers, Alex. Schmidt, Hammarsten, Arthus, recognised the stage prothrombin which was converted into thrombin by calcium salts. According to Morawitz, the precursors are thrombogen, which exists as such in the plasma, and thrombokinase, which is produced by the corpuscles, or platelets, or may come from other tissues. Thrombogen and thrombokinase give thrombin in the presence of calcium salts. The evidence for the presence of thrombokinase was obtained from experiments with birds' blood; if it be carefully collected and the corpuscles separated without damage, it does not clot, but it clots on adding damaged corpuscles, or a scraping of muscular tissue.

Thrombin has been considered to be a ferment, or enzyme, but the observations of Howell and Rettger show that a solution of thrombin, if obtained almost free from protein, is stable to heat. Enzymes are generally recognised to be more or less easily destroyed by heating.

The following scheme represents the processes which occur in the formation of fibrin :—

$$\text{Prothrombin}\begin{cases}\text{Thrombogen}\\\text{Thrombokinase}\\\text{Calcium salts}\end{cases}\left.\begin{array}{c}\text{Thrombin}\\\\\text{Fibrinogen}\end{array}\right\}\text{Fibrin} + \text{(Soluble globulin ?)}$$
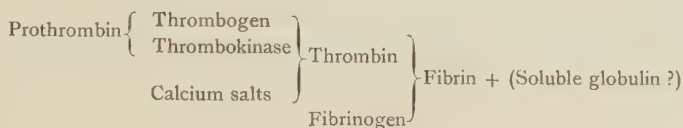
For the elucidation of the above factors in the scheme of blood clotting it was necessary to prevent the blood from clotting, to prepare fibrinogen in a pure state from non-coagulated blood, to prepare a solution of fibrin ferment, or thrombin, and to determine the factors leading to the formation of thrombin.

Prevention of Clotting of Blood.

Blood may be prevented from clotting by collecting it in various salt solutions when it is drawn, e.g. sodium sulphate, magnesium sulphate, potassium oxalate, sodium fluoride, sodium citrate. Salt plasmas are thus obtained.

Clotting may also be hindered, or prevented, by keeping the drawn blood at a low temperature (0°), or by adding leech extract to it immediately after it is drawn.

If peptone be injected into the circulation, or if leech extract or the active principle of leech extract, termed hirudin, be injected, the blood when drawn does not coagulate.

The Interaction of Calcium Salts, Prothrombin and Fibrinogen.

The formation of fibrin from fibrinogen by the action of thrombin, and the formation of thrombin by the action of calcium salts upon prothrombin is easily observed by the following experiments with oxalate plasma :—

Oxalate Plasma.—This is obtained by collecting 9 parts (900 c.c.) of blood in 1 part (100 c.c.) of 1 per cent. potassium oxalate solution, shaking well during the mixing. The plasma is then separated from the corpuscles by centrifugalising.

For the study of each detail in clotting, the plasma should be quite free from blood corpuscles, as their stromata may serve as source of thrombin.

(1) On diluting with 5 volumes of water, no clotting occurs at 40°.

(2) On diluting and adding a few drops of calcium chloride and warming to 40°, clotting occurs.

(3) On diluting and adding a drop of serum, freed from calcium salts by precipitation with oxalate, and warming to 40°, clotting occurs.

The plasma contains fibrinogen, but the formation of thrombin has been prevented by the absence of calcium salts. On their addition, thrombin is formed and clotting takes place.

Serum contains thrombin, and hence, when added free from calcium salts to plasma free from calcium salts, clotting occurs.

Fibrinogen.

Fibrinogen can be prepared from oxalate plasma by precipitation with sodium chloride, or ammonium sulphate.

*   It is precipitated from oxalate plasma by quarter-saturation with ammonium sulphate, thus :—

Every 12 c.c. of oxalate plasma are diluted with 30 c.c. of water and 20 c.c. of saturated ammonium sulphate are added. The precipitate is filtered off, washed with quarter-saturated ammonium sulphate, dissolved in water and reprecipitated.

Fibrinogen gives the colour reactions, coagulation reactions and precipitation reactions of proteins.

It is a globulin, being soluble in dilute salt solutions but insoluble in water. It is precipitated from solution by salts, but less than complete saturation (half-saturation) with sodium chloride and less than half-saturation (quarter-saturation) with ammonium sulphate throws it out of solution. It is, therefore, an atypical globulin.

Its temperature of heat coagulation in a dilute salt solution is 56°.

It is converted into insoluble fibrin by thrombin.

*   A solution of fibrinogen in water [1] may clot at 40° on adding calcium chloride, as it will be contaminated with thrombokinase and thrombogen.

It is converted into fibrin, if a drop of serum, or thrombin solution, be added.

Fibrin.

Fibrin is not usually prepared from fibrinogen, but directly from blood. The blood, when drawn, is immediately whipped with a bundle of twigs. Threads of fibrin collect on the twigs ; they are removed, placed in a muslin bag, and washed with running water.

The freshly prepared threads of fibrin are nearly colourless ; on drying by exposure to the·air, they form a brownish mass. The fresh threads are best preserved in glycerol, which is easily removed by washing.

Fibrin is insoluble in water, salt solutions, cold dilute solutions of acids and alkalies. It dissolves, on warming, in dilute acid, or alkali, but undergoes hydrolysis into its derivatives.

[1] It dissolves in water as sufficient salt is still present with it.

A suspension of fibrin in water will give the colour reactions for proteins. The solution in acid, or alkali, will behave like metaprotein, or proteoses and peptone, depending on the length of time the solution has been heated. It will be precipitated by heavy metals and alkaloidal reagents.

Thrombin.

Thrombin is not present in blood plasma, but is formed in the process of clotting : it will therefore be present in the serum and upon the fibrin.

(1) *Preparation from Serum, or Defibrinated Blood.*

1 volume of serum, or defibrinated blood, is mixed with 15-20 volumes of alcohol and the mixture is allowed to stand for some weeks. The precipitate, which is formed, is filtered off, washed with alcohol and dried in a desiccator. It contains thrombin, which is extracted by water (Schmidt).

(2) *Preparation from Fibrin.*

It is best to use fibrin which has been obtained from blood diluted with 10 volumes of water and which has been washed with water. This fibrin is preserved in weak alcohol. A solution of thrombin is obtained by extracting the fibrin with 8 per cent. sodium chloride solution (Gamgee).

SERUM.

Blood serum contains three coagulable proteins [1] :—

Globulin, or euglobulin—insoluble in water
Pseudoglobulin—soluble in water
⎱ precipitated by half-saturation with ammonium sulphate.

Albumin—soluble in water, precipitated by complete saturation with ammonium sulphate.

A nucleoprotein is also present in small quantities. Serum also contains the other constituents of blood.

Defibrinated blood contains the same proteins and the red and white corpuscles in addition. These are removed by centrifuging and serum, probably more or less pigmented by hæmoglobin, results.

Serum, free from hæmoglobin, is a faintly yellow fluid of sp. gr. 1030 and alkaline to litmus.

A dilute solution of serum (1-10 of water) acidified with a drop of acetic acid coagulates on heating at 70-80°, chiefly between 73-75°.

It gives all the general reactions for proteins.

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[1] Haslam, Biochem. J., 1913. vol. vii.

Globulin, or Euglobulin.

Preparation.

Globulin can be prepared by dialysing serum, or by acidifying it with acetic acid and passing carbon dioxide through it. The precipitate is dissolved in dilute salt solution and again thrown out, preferably by dialysis, or by sodium chloride.

Serum is half-saturated with ammonium sulphate. Euglobulin and pseudoglobulin are precipitated. The precipitate is treated with saturated sodium chloride solution. The euglobulin is insoluble ; the pseudo-globulin is soluble.

The euglobulin can be purified by dissolving in dilute salt solution and precipitating with sodium chloride.

The globulin is obtained in a coagulated state by treating the precipitate with alcohol and ether, or it may be obtained by dissolving in dilute salt solution, acidifying and boiling. The coagulum is washed with boiling water and dried with alcohol and ether.

Properties.

The uncoagulated protein is a white amorphous substance, insoluble in water, but soluble in dilute salt solutions. It is precipitated by saturating its solution with sodium chloride, or magnesium sulphate, or by half-saturation with ammonium sulphate.

It gives the general reactions of the proteins and is a typical globulin.

The coagulated protein is insoluble, but dissolves on warming in dilute acids and alkalies, undergoing conversion into derivatives.

Pseudoglobulin.

Preparation.

The pseudoglobulin, dissolved in sodium chloride solution (above), is thrown out again by half-saturation with ammonium sulphate. It is dissolved in sodium chloride solution and the solution dialysed.

In a coagulated state it is obtained by precipitating with alcohol and drying with alcohol and ether, or by acidifying the solution and boiling, drying the coagulum after washing with alcohol and ether.

Properties.

The uncoagulated protein, if its solution in water be evaporated *in vacuo*, will form an amorphous glassy mass of a yellow to brown colour. The coagulated protein is an amorphous white, or nearly white, powder.

Pseudoglobulin is not a typical globulin, as it is soluble in water and in saturated sodium chloride solution.

Its solution gives the general reactions for proteins.

Serum Albumin.

Preparation.

Serum albumin remains in solution after the globulins have been precipitated by half-saturation with ammonium sulphate. It is precipitated by complete saturation of the filtrate with ammonium sulphate.

It is purified by dissolving in water, half-saturating with ammonium sulphate, filtering and completely saturating with ammonium sulphate and repeating the process several times. The final solution is dialysed to remove ammonium sulphate and the coagulated protein obtained either by acidifying and boiling, or by precipitation with alcohol, or it may be evaporated *in vacuo*.

Crystalline Serum Albumin.

Crystalline serum albumin can be prepared in a similar way to crystalline ovalbumin.

The globulins are removed by slowly adding an equal volume of saturated ammonium sulphate to serum and stirring thoroughly; after 4 or 5 hours they are filtered off. The filtrate is treated with ·2N sulphuric acid until there is a permanent turbidity (10-14 c.c. per 100 c.c.). Crystals separate out as the solution stands. They are filtered off, dissolved in water and recrystallised by adding acid and ammonium sulphate. This is repeated several times.

Coagulated protein is obtained from it by pouring its solution in water into alcohol, washing the coagulum with water and drying with alcohol and ether.

Properties.

Uncoagulated serum albumin, obtained by evaporation of the dialysed solution, forms an amorphous yellowish mass. It is soluble in water and its solution is coagulated by heating when acidified with acetic acid. It shows all the general reactions of the proteins.

Coagulated serum albumin forms a white amorphous powder which is insoluble in water and salt solutions. It is dissolved by dilute acids and alkalis on warming and is hydrolysed into derivatives. It is more resistant to acids than egg-albumin.

REACTION OF BLOOD.

Blood is alkaline in reaction to litmus and methyl orange; it is acid to phenolphthalein. The alkalinity is due chiefly to disodium phosphate and corresponds to ·4 per cent., or ·1N, sodium hydroxide.

The alkalinity to litmus is easily seen by placing a drop of blood upon a piece of neutral glazed litmus paper and in 10-30 seconds washing off the blood with distilled water; a blue mark remains.

SPECIFIC GRAVITY OF BLOOD.

The specific gravity of human blood is usually from 1054-1060, but values of 1040 and 1065 have been observed. The specific gravity of defibrinated blood usually varies from 1050-1055.

The specific gravity can be determined by weighing against an equal volume of water.

It is most readily determined with fair accuracy by Hammerschlag's method. A mixture of chloroform and benzene, or ligroin, of specific gravity about 1055 is prepared. A drop of blood is blown out from a capillary tube below the surface of the mixture; it assumes a spherical shape and will float, or sink. Benzene, or chloroform, is added until the mixture is such that the drop neither sinks nor rises. The specific gravity of the mixture is ascertained by means of a hydrometer, or by means of specific gravity beads. It is the same as that of the blood.

* The specific gravity may also be determined by Roy and Lloyd-Jones' method. A series of solutions of sodium sulphate of specific gravity from 1035-1070 are prepared. Portions of these solutions are placed in glass thimbles, or small test tubes. The blood from the finger (or defibrinated blood) is drawn up into a capillary pipette which is made from a glass tube and bent at right angles; the pipette is conveniently furnished with an india-rubber cap. A drop of blood is ejected from the pipette in the centre of one of these solutions. It rises or sinks; drops are ejected into neighbouring solutions. In one of them it will remain stationary. The specific gravity of this solution will be the same as that of the blood.

THE RED BLOOD CORPUSCLES.

The number of red blood corpuscles in the blood of animals varies in health considerably, thus it is roughly :—

In man	5,000,000	
dog	6,500,000	
cat	9,000,000	
pig	6,000,000-8,000,000	in 1 cubic millimetre.
horse	7,700,000	
ox	6,000,000-8,000,000	
sheep	10,000,000	
goat	13,000,000-18,000,000	

Variations in the number of corpuscles occur in man when he lives at different altitudes, more corpuscles being present at high altitudes. Variations in the number also occur pathologically. A variation of 100,000 in 5,000,000 is of no physiological importance.

Dog's red corpuscles have the following composition (Abderhalden) :—

Water, 64·4 per cent.   Hæmoglobin, 32·75 per cent.
Solids, 35·6   ,,   Proteins,   9·92   ,,

Enumeration of the Red Blood Corpuscles.

The number of red blood corpuscles in blood is counted with a hæmocytometer. A drop of blood is diluted and put into a special cell, the bottom of which is ruled with a number of minute squares. By microscopic observation the number of corpuscles on several squares are counted. Several patterns of hæmocytometer are commonly used.

The Thoma-Zeiss Hæmocytometer.

The apparatus (Fig. 49) consists of (1) a glass slide upon which is
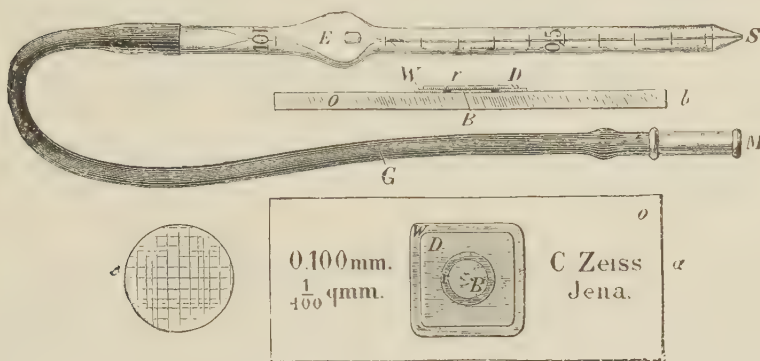
FIG. 49.

fastened a square glass cell : a circular disc ruled in squares of $\frac{1}{400}$ sq. mm. area is cemented to the floor of the cell. The cell is covered with a cover slip. The height of the cover slip from the bottom of the cell is ·1 mm. A volume of liquid occupying ·1 × $\frac{1}{400}$ or $\frac{1}{4000}$ cmm. is above each square ; (2) a graduated pipette with a bulb containing a glass bead. The divisions bear an exact ratio to the volume of the blood.

The second drop of fresh blood from the finger, or ear, cleaned with acetone, and obtained by pricking with a lancet, is sucked up in the pipette to the mark ·3, ·5 or 1. The pipette is wiped and diluting fluid [1] is sucked up to the mark 101 whilst the pipette is continuously rotated between the finger and thumb so as to ensure mixing. The pipette is closed with the fingers without removing the rubber tube and the contents are shaken for 1 minute. Three or four drops are blown out of the pipette and then a minute drop is placed on the centre of the ruled disc. The cell is covered with the cover slip, avoiding any escape of the liquid into the portion around the disc. The number of corpuscles in 40 squares is counted. Corpuscles on the top and right side do not belong to the square. The counting

[1] Hayem's fluid = 5 gm. $HgCl_2$ + 5 gm. Na_2SO_4 + 1 gm. NaCl + 200 c.c. H_2O, coloured with methyl violet.

should be in the order five left to right, five right to left in next row, five left to right in next row, and so on.   It is advisable to note the totals for each 5 or 10 squares.

The dilution of the blood is as the volume of blood taken to the total volume of the mixture, e.g. :—

$$·3/100 \qquad ·5/100 \qquad 1/100$$

i.e. it is $\dfrac{100}{·3}, \dfrac{100}{·5}, \dfrac{100}{1}.$

The volume of the liquid above the squares is $\frac{1}{4000}$.

Hence the number of corpuscles per cubic millimetre of blood is

$$\dfrac{4000 \times \text{dilution} \times \text{total corpuscles counted}}{\text{No. of squares counted}}$$

The error is 5 per cent. when 16 squares are counted, 2 per cent. for 100 and 1 per cent. for 400.

LAKING OF BLOOD. HÆMOLYSIS.

Blood, or defibrinated blood, is opaque even in very thin layers, but becomes transparent when the hæmoglobin, which is normally contained in the red blood corpuscles, is discharged from them.   The blood is said to be laked, or hæmolysed.

Blood may be laked in several ways :—

(*a*) By osmosis, i.e. by diluting it with 2 or 3 volumes of water, or by adding blood to excess of water.   A solution is obtained which is transparent and has a bright red colour.   In the process of osmosis, water passes into the corpuscles, bursts them and liberates the hæmoglobin. This does not occur when blood is mixed with 0·9 per cent. sodium chloride solution, since the contents of the corpuscles and the saline solution have the same osmotic pressure, or are isotonic.

More concentrated solutions of salt produce crenation of the corpuscles, since water is withdrawn from them.   Such solutions are hypertonic ; more dilute solutions are hypotonic.

(*b*) By the action of weak alkali.   Blood is mixed with ·9 per cent. sodium chloride solution and a few drops of alkali are added.   The blood becomes transparent.

(*c*) By the action of chloroform, or ether.   Blood diluted with ·9 per cent. salt solution becomes transparent if a few drops of ether, or chloroform, are added and mixed with it.

(*d*) By alternate freezing and thawing.   The blood on subsequent dilution with ·9 per cent. sodium chloride solution will be transparent.

* (*e*) By the action of urea. Blood mixed with an isotonic solution of urea becomes transparent.

(*f*) By glucosides, such as saponin.

(*g*) By bacterial toxins, snake venoms and by the blood serum of another animal.

* THE CATALYTIC ACTION.

Blood contains a very active catalytic enzyme, or catalase, which is sometimes termed hæmase. An evolution of oxygen occurs on adding a little hydrogen peroxide to defibrinated blood.

* THE PEROXIDASE ACTION.

Blood, owing to the presence of hæmoglobin, gives very marked peroxidase reactions (p. 412).

(*a*) *With Guaiacum.*

* One drop of blood is diluted considerably with water (about 10 c.c.), one-half of its volume of hydrogen peroxide (20 vols. per cent.) is mixed with the solution and upon the surface is floated some fresh tincture of guaiacum, or better, a 1 per cent. alcoholic solution of guaiaconic acid. A blue colour gradually develops above the resinous ring at the point of contact and diffuses into the clear zone above it. The blue colour ultimately disappears. The blood should not be left in contact with the peroxide for even as long as 5 minutes before adding the guaiaconic acid solution.

More usually a few c.c. of 1 per cent. guaiaconic acid in equal parts of alcohol and water are mixed with half the volume of 3 per cent. hydrogen peroxide and the blood solution added. The blue colour develops and disappears.

This reaction succeeds when blood is diluted 1 in 10,000. It is not due to any enzyme, as it is given by boiled solutions of blood. It depends upon the presence of iron, as it is not given by hæmatoporphyrin, but is given by all the derivatives of hæmoglobin which contain iron. It may be termed a pseudo-peroxidase reaction (Buckmaster).

* (*b*) *With Benzidine.*

3 or 4 milligrams of benzidine are dissolved in 2 c.c. of glacial acetic acid ; 10 drops are put into a clean test tube and mixed with 30 c.c. of 3 per cent. hydrogen peroxide. No colour should appear. A blue-green colour is formed on adding a dilute solution of blood. This reaction has a delicacy of at least 1 in 100,000 depending on the method of carrying out the reaction.

HÆMOGLOBIN AND ITS DERIVATIVES.

Hæmoglobin and its derivatives show typical absorption bands when their solutions are examined with a spectroscope. The formation of the derivatives is thus readily observed and they are thus easily identified.

Construction of a Spectroscope.

A spectroscope consists essentially of 3 parts, a collimator, or tube furnished with a slit at one end and a convex lens at the other, so as to produce a beam of parallel rays, a prism and a telescope to focus the light for observation. There is usually also a scale of wave-lengths attached to the telescope so as to be able to note the position of any absorption bands. The beam of white light of parallel rays, which reaches the prism from the collimator, is resolved by the unequal refraction of its constituents at each bent surface into a band of light of several colours. This band of light is termed a spectrum, the colours being red, orange, yellow, green, blue, violet and indigo.

A spectrum obtained from a beam of sunlight shows a series of vertical dark lines—the Fraunhofer's lines. Thus, in the red region three lines are seen; they are known as the A, B, C lines; in the yellow region one line, the D line; in the green three, the E, *b*, F lines; in the violet two, the G and H lines. These dark lines are due to the absorption of light in these regions of the spectrum by the passage of the light through certain volatile substances in the sun's atmosphere. The D line is due to sodium vapour as can be shown by passing ordinary light through sodium vapour; a line, or rather two lines very close together, are seen in the D position. The other lines are due to the vapours of other elements. The colour of light after passing through a coloured liquid is the colour which is not absorbed by the liquid.

When light is passed through a solution of hæmoglobin it is absorbed in certain regions. These regions become visible when the light is passed through a prism. They are the absorption bands.

In an ordinary spectroscope the field of vision is at an angle to the original source of light, but for convenience a direct vision spectroscope is used. Such a spectroscope consists of a series of prisms of crown and flint glass. The light which enters is refracted at each prism and finally emerges in the same direction as the original beam of light.

An absorption band has a certain width and a point of maximum absorption which does not alter with different concentrations of the solution; this point of maximum absorption is determined in very accurate work and noted on the line of wave-lengths. The width of the band alters with different concentrations and is therefore not registered. For ordinary work the absorption bands visible to the eye only are noted, but for detailed work the absorption bands in the ultra-violet region, which are not visible to the eye are also determined by photographing on special plates.

The Absorption Spectra of Hæmoglobin and its Derivatives.

A solution of 1 volume of defibrinated blood, or blood, is gradually diluted with 30 volumes of water and examined with a spectroscope. The concentrated solution appears dark red to the naked eye, but light will be found to pass through the red region of the spectrum

between the Fraunhofer's lines C and D. On diluting, light will pass through the green region and between the red and green regions a dark absorption band will be seen. On diluting further, this band becomes resolved into two, the one lying nearer D being narrower than the other. It is immediately to the right of the sodium, or D, line, as can be seen by introducing a little sodium chloride into the flame when examined by gaslight. At the same time light will pass through the violet region.

It should be noted at what dilution of the blood these two bands are first clearly visible, so that this dilution can be used for the examination of the derivatives.

(a) **Oxyhæmoglobin,** HbO_2.

The two bands as seen above are characteristic of oxyhæmoglobin.

Very dilute solutions of oxyhæmoglobin, yellowish-red in colour to the naked eye, still show the two characteristic bands.

(b) **(Reduced) Hæmoglobin,** Hb.

The most remarkable property of hæmoglobin is its power of absorbing a molecule of oxygen and its power of giving it up again. Oxyhæmoglobin and hæmoglobin exist side by side in circulating blood; oxyhæmoglobin preponderates in amount in arterial blood, hæmoglobin usually in venous blood; the difference in the red shade of two kinds of blood shows which preponderates; the bright red colour is that of oxyhæmoglobin, the dull red colour is that of hæmoglobin. Blood kept in the absence of oxygen becomes dull red and will contain hæmoglobin. Hæmoglobin is characterised by a single absorption band.

Solutions of hæmoglobin can be artificially prepared by the action of reducing agents upon blood, such as alkaline solutions of ferrous salts (Stokes' reagent), ammonium sulphide, sodium thiosulphate, hydrazine; thus

(i) A few drops of ammonium sulphide are added to a solution of oxyhæmoglobin and the solution is gently warmed to about 50°. The colour becomes darker and, on examination with a spectroscope, a single broad absorption band will be seen between D and E.

(ii) Two or three drops of Stokes' reagent [1] are added to a solution

---

[1] Stokes' reagent consists of ferrous sulphate, tartaric acid and ammonia. It is prepared by dissolving 3 gm. ferrous sulphate in cold water, adding a cold solution of 2 gm. tartaric acid and then making the mixture up to 100 c.c. Strong ammonia is then added till the precipitate first formed is redissolved. The solution rapidly absorbs oxygen and must therefore be freshly prepared. The solution of ferrous sulphate and tartaric acid can be kept for some time; the ammonia is therefore added when the reagent is required for use.

of oxyhæmoglobin.   It acts more rapidly than ammonium sulphide and need not be warmed.   The single absorption band of hæmoglobin is seen with a spectroscope.

*   *Conversion of Hæmoglobin into Oxyhæmoglobin.*

On shaking up the solution of hæmoglobin with air, it is converted into oxyhæmoglobin and two bands become visible.   If excess of reducing agent has been added, the two bands will disappear and the solution will show the single band of hæmoglobin.

*   ## (c) **Carboxyhæmoglobin**, COHb.

Hæmoglobin combines with carbon monoxide forming carboxyhæmoglobin.   Carboxyhæmoglobin differs from oxyhæmoglobin in being more stable; it can only be dissociated by prolonged treatment with oxygen.   It is formed in cases of coal-gas poisoning by the action of the carbon monoxide in the gas.   Owing to its stability, cases of coal-gas poisoning are generally fatal.

A stream of coal gas is passed through a solution of defibrinated blood.   It assumes a cherry-red colour as compared with the yellowish-red of oxyhæmoglobin, due to the smaller absorption of light in the blue and violet regions.

Spectroscopic examination will show two bands like those of oxyhæmoglobin, but they are situated slightly nearer the violet end of the spectrum.   The difference in position can only be determined by the use of a spectroscope with a scale of wave-lengths.

*   *Differentiation between Carboxyhæmoglobin and Oxyhæmoglobin.*

Oxyhæmoglobin is reduced to hæmoglobin by reducing agents, but carboxyhæmoglobin is not reduced.   If therefore some ammonium sulphide be added to a solution of carboxyhæmoglobin and the solution be gently warmed, no change is noticed in the absorption bands.

The blood in cases of suspected coal-gas poisoning must always be tested by the reduction.

Solutions of carboxyhæmoglobin (saturated with carbon monoxide) become light red in colour on treatment with an equal volume of 40 per cent. caustic soda; a red precipitate is deposited.   Solutions of oxyhæmoglobin give a brown coloration and brownish-black precipitate.

Tannic acid gives a red precipitate with carboxyhæmoglobin solutions, a greenish-brown precipitate with oxyhæmoglobin.

On boiling a pure solution of carboxyhæmoglobin, a red precipitate is obtained; oxyhæmoglobin solutions give a brown precipitate.

Methæmoglobin, HbO.

Solutions of oxyhæmoglobin on exposure to the air become brown in colour and on spectroscopic examination show an absorption band in the red as well as the two bands of oxyhæmoglobin. The substance giving the extra band was called methæmoglobin by Hoppe-Seyler in 1864.

Methæmoglobin is produced by the action of numerous substances upon hæmoglobin—such as nitrates, chlorates, permanganates, hydrogen peroxide, nitrobenzene, pyrogallol, etc. It is most conveniently prepared by the action of potassium, or sodium, ferricyanide, thus :—

A dilute solution of blood is treated with a few drops of a strong solution of potassium ferricyanide. The solution becomes reddish-brown in colour. On examination with a spectroscope, the solution shows an absorption band to the red side of D and the blue end is markedly absorbed. There is also a faint band in the green-blue region. On dilution, the two bands of unchanged oxyhæmoglobin may appear.

The absorption band in the red is most characteristic for methæmoglobin.

Conversion of Methæmoglobin into Hæmoglobin and Oxyhæmoglobin.

Methæmoglobin is converted by reducing agents, e.g. ammonium sulphide, into hæmoglobin, and, on shaking with air, oxyhæmoglobin is formed.

Solutions of methæmoglobin may thus be distinguished from solutions of hæmatin.

The Change of Oxyhæmoglobin into Methæmoglobin.

When oxyhæmoglobin is converted into methæmoglobin, oxygen is evolved.

The evolution of oxygen in the conversion of oxyhæmoglobin into methæmoglobin can be readily observed :—

An equal volume of water is added to a little defibrinated blood in a test tube and warmed to 50°. An equal volume of potassium ferricyanide solution is mixed with this solution. If the tube be inclined for a short time, the evolution of bubbles of oxygen will be seen.

The work of Letsche and of Buckmaster and of Nicloux upon the action of hydrazine on oxyhæmoglobin and methæmoglobin shows that methæmoglobin contains half as much oxygen as is present in oxyhæmoglobin. Methæmoglobin has probably the formula $Hb - O$ and the conversion of oxyhæmoglobin into methæmoglobin may be

$$Hb\begin{cases} O \\ | \\ O \end{cases} + O = Hb = O + O_2$$

or

$$Hb\begin{cases} OH \\ OH \end{cases} + O_2 = Hb = O + H_2O + O_2.$$
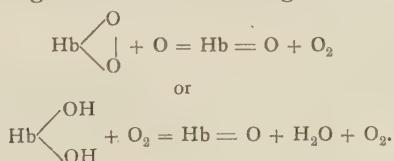
Hæmatin.

*

Hæmoglobin is decomposed by the action of dilute alkalies, or dilute acids, into hæmatin and globin; also by digestion with pepsin and hydrochloric acid. Hæmatin is insoluble in water, but soluble in acids or alkalies, giving solutions which are known as acid hæmatin and alkaline hæmatin. In alkaline solution, hæmatin can be reduced by Stokes' reagent, or ammonium sulphide, to hæmochromogen, or reduced alkaline hæmatin. These changes can also be seen with a solution of defibrinated blood.

*

Acid Hæmatin.

A quarter of a volume of 33 per cent. acetic acid is mixed with some diluted blood (1 : 5) and warmed in a water-bath to 40 or 50° for 5-10 minutes. The solution becomes brown. On diluting a portion with water and examining with a spectroscope, an absorption band in the red between C and D will be seen. This is characteristic of acid hæmatin; it resembles the absorption spectrum of methæmoglobin. Its position depends on the amount of acid present in the solution; it is nearer C with more acid.

*

Conversion into Hæmochromogen.

The solution is made faintly alkaline with dilute caustic soda and filtered. Stokes' reagent, or ammonium sulphide, is added to the filtrate. The two characteristic bands of hæmochromogen in the green region are seen with a spectroscope.

Acid Hæmatin in Alcohol.

Acid hæmatin is soluble in alcohol. A solution is most readily obtained by adding sufficient alcohol to blood to precipitate the proteins. The precipitate is warmed with alcohol containing 1 per cent. of sulphuric acid. A dark-brown solution of acid hæmatin showing the absorption bands is obtained.

Acid Hæmatin in Ether.

Acid hæmatin dissolves in ether. An ethereal solution is prepared by adding half the volume of glacial acetic acid to defibrinated blood and an equal volume of ether and mixing thoroughly. The ethereal solution of acid hæmatin rises to the surface. On pouring it off into a clean vessel and examining it with a spectroscope, it will show the characteristic band in the red region and a broader band between D and F. This band is resolved into two bands, if the solution be diluted with acid ether (1 part of glacial acetic acid to 2 parts of ether). There is a narrow band in the light green to the red side of E and a broader, darker one in the green. A fourth very faint band may be seen on the violet side of D.

*

Alkaline Hæmatin.

Dilute defibrinated blood (1 : 5) is mixed with half its volume of alcoholic caustic soda solution and heated gradually nearly to the

boiling-point. The colour becomes brown. On cooling and shaking with air (reducing substances are formed by the action of alkali and may reduce the hæmatin to hæmochromogen) and examining the solution with a spectroscope, a broad but very faint band will be seen to the red side of D, extending a short distance towards the violet side. The blue end of the spectrum is considerably absorbed.

Alkaline Hæmatin in Alcohol.

Alkaline hæmatin is soluble in alcohol and is prepared by mixing a little defibrinated blood with solid potassium carbonate so as to form a paste and evaporating to dryness on the water-bath. The dry residue is boiled in a flask on a water-bath with alcohol. The filtered solution shows the absorption band of alkaline hæmatin. It is more distinct than with the aqueous solution.

* ### Conversion into Hæmochromogen.

A few drops of ammonium sulphide are added to the alkaline solution and warmed. The solution becomes red in colour and on spectroscopic examination shows two bands in the green, the one nearer D being very prominent and sharply defined, the other being much fainter.

In very dilute solutions only the one band is seen, but the absorption of light here is very great. A solution of oxyhæmoglobin, where the absorption bands can scarcely be seen, on conversion into hæmochromogen, will show this single band.

The conversion of hæmoglobin into hæmochromogen is a very delicate test for blood stains ; the stain is heated with 1 per cent. caustic soda, the solution is cooled and filtered and then reduced. Examination with the spectroscope shows one, or both, absorption bands.

* ### Hæmin.

Hæmatin forms a hydrochloride which is insoluble in acetic acid and is termed hæmin. It crystallises in blue-black prisms and is very easily prepared as a microscopical preparation :—

A drop of blood is placed on a glass slide and allowed to dry, or it is dried by gently heating over a flame. The dry residue is scraped into a little heap and a drop of glacial acetic acid added to it from the end of a glass rod. It is rubbed up into a paste and a little is put on a

FIG. 50.—Hæmin crystals.

clean slide. A drop of glacial acetic acid is added, it is covered with

31

a cover slip and heated over a small flame till the acid just begins to boil. The slide is allowed to cool and examined with a microscope. Small black, or brownish-black, crystals of hæmin are seen as shown in Fig. 50. If no crystals are visible, the glass slide is again heated and the heating may be repeated two or three times.

Hæmatoporphyrin.

Hæmoglobin and hæmatin are decomposed by the action of strong acids and converted into hæmatoporphyrin.

Hæmatoporphyrin is free from iron, whereas hæmatin still contains this element. The removal of the iron from hæmatin in the absence of reducing agents is accomplished only with difficulty and is only effected by strong reagents, such as concentrated sulphuric acid, glacial acetic acid saturated with hydrobromic acid, or hydrochloric acid, and heated to 130° in a sealed tube. The hæmatoporphyrin obtained by the action of sulphuric acid is hæmatoporphyrin anhydride.

Hæmatoporphyrin is more readily formed from reduced hæmoglobin by the action of moderately concentrated hydrochloric acid.

The iron is also readily removed from hæmochromogen by dilute acids, and reducing agents of an acid nature easily set free hæmatoporphyrin from hæmatin. Hence the stability of iron in hæmatin is dependent on the presence of oxygen.

It is very probable that the cells of the body deal with the blood pigment in the reduced condition, which, as seen above, is easily changed into hæmatoporphyrin. Its occurrence in the urine in cases of hæmatoporphyrinuria can be thus accounted for. Alkaline hæmatoporphyrin occurs in urine.

Hæmatoporphyrin is soluble in dilute acids and alkalies giving solutions of acid and alkaline hæmatoporphyrin.

Acid Hæmatoporphyrin.

(*a*) A few drops of blood are mixed with 5 c.c. of concentrated sulphuric acid. A purple solution is obtained, which, when examined with the spectroscope, will show two well-marked absorption bands. One of the absorption bands lies in the orange between C and D ; the other, which is broader and darker, lies in the yellow-green between D and E. The solution may be diluted with glacial acetic acid, if necessary.

(*b*) Blood which has stood for 2-3 days in the cold, i.e. has become reduced by standing, is treated with one-third of its volume of concentrated hydrochloric acid and filtered. The mass of hæmatopor-

phyrin and precipitated proteins is extracted with alcohol ; the alcoholic filtrate shows the spectrum of acid hæmatoporphyrin (Laidlaw).

* *Alkaline Hæmatoporphyrin.*

(*a*) The sulphuric acid solution of hæmatoporphyrin is poured into excess of distilled water. The solution is cooled and neutralised with caustic soda. A pigmented precipitate containing most of the hæmatoporphyrin separates out. Sodium acetate makes the precipitation more complete. The precipitate is filtered off and dissolved in dilute caustic soda. The solution of alkaline hæmatoporphyrin so obtained shows, when examined with the spectroscope, four absorption bands, a narrow one in the red, a broader and darker one in the green, a third in the green extending to the violet side of E and a fourth at the junction of the blue and green.

(*b*) On rendering the hydrochloric acid solution just alkaline with ammonia, the spectrum of alkaline hæmatoporphyrin may be observed. It is, however, best seen if the alcoholic solution be evaporated to dryness on the water-bath and the pigment dissolved in dilute ammonia.

* ## ESTIMATION OF HÆMOGLOBIN.

Most of the methods which have been devised for the estimation of hæmoglobin depend upon the comparison of the colour of blood with that of carefully prepared standard colours. These standard colours are either various shades of red painted on paper, or coloured glass, or they are a standard colour in solution made from a suitably diluted normal blood. The standard solution may be blood, treated with carbon monoxide to give carboxyhæmoglobin, diluted to a fixed amount.

The amount of hæmoglobin may also be determined by ascertaining the oxygen capacity of the blood and indirectly by an enumeration of the red blood corpuscles, if each corpuscle contains the normal amount.

A. Colorimetrically.

* (1) *Tallquist's Method.*

In this method the colour of a drop of blood is matched against a series of red spots on paper. These spots have colours varying from light to dark red and represent percentages from 10 to 100. They are arranged in a series and by their side is a circular opening in the paper. A drop of blood from the finger is touched with a piece of white blotting-paper, or filter paper, and allowed to diffuse through the paper so as to give an even stain. As soon as the blood is dry, the stain is viewed through the openings in the paper against the standards and the colours are matched. The percentage of hæmoglobin in the blood corresponds with that of the standard. As the standards do not

show every unit, a very close approximation cannot be made, e.g. the blood stain may be deeper in colour than the 95 per cent. standard, but paler than the 100 per cent. one. The method gives good average results, but is not very accurate.

(2) *Von Fleischl's Method.*

In this method an apparatus (Fig. 51) consisting of a mirror (*R*), a coloured glass wedge (*K*) and a circular cell (*G*) with two partitions (*a, a'*) is used. The mirror has a white reflecting surface (*S*) and is adjusted to send light through the coloured wedge and through the two compartments of the cell. One of the compartments of the cell is filled with water; in the other compartment is placed a small amount of water; 20 cmm. of blood are collected from the finger in a specially graduated capillary pipette and ejected into the water in this compartment, which is then completely filled with water. The volume of the compartment is such that the blood is diluted 100 times. The colours are observed and the wedge is moved by means of a screw (*T*) until the colours match. The graduated scale (*P*) on the instrument gives the percentage of hæmoglobin.
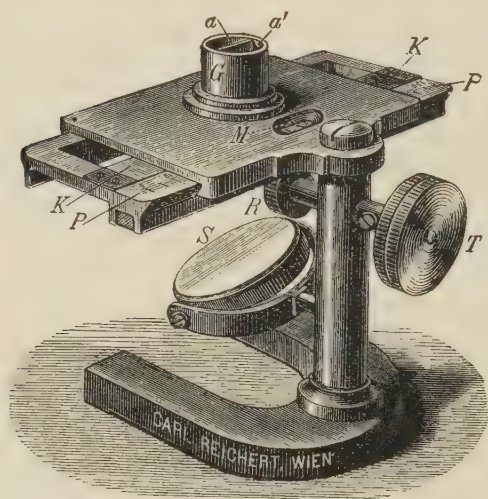
Fig. 51.

* (3) *Haldane's Method.*

Haldane uses for comparison a standard solution of ox blood, or sheep's blood, saturated with carbon monoxide. This standard is a 1 per cent. solution of defibrinated ox blood saturated with coal gas. It has an oxygen capacity, determined by Haldane's ferricyanide method, of 18·5 per cent.; the carbon monoxide capacity is the same. Since 1 gm. of hæmoglobin combines with 1·34 c.c. of oxygen at N.T.P., it corresponds to 13·8 per cent. of hæmoglobin. This is the same as that of adult males. Women's blood averages 11 per cent., children's blood 13 per cent. In determining the percentage of hæmoglobin in these cases, $\frac{1}{8}$ should be added for women's blood and $\frac{1}{3}$ for children's. The standard, kept in the dark, will remain unaltered for years.

* The standard is contained in a small sealed tube. The comparison is made in a similar tube with 20 cmm. of blood which is treated with carbon monoxide and diluted with water until it has the same tint. This tube is graduated in percentages, so that the value is directly observed.

The apparatus necessary for the estimation is the standard tube (D), a companion tube graduated in percentages (C), a lancet for pricking the finger (F), a pipette to measure 20 cmm. of blood (B), a tube to pass carbon monoxide, or coal gas, into the vessel and a bottle of distilled water saturated with coal gas with a drop pipette for gradually diluting the blood (A). The items are shown in Fig. 52.

The process of estimation is carried out as follows :—

The water is saturated with coal gas by attaching the cap of the special tube for the purpose to a gas burner and passing in the gas. The water is shaken several times with the gas so that it becomes saturated.

In the small tube, which is graduated in percentages from 0 to 100 or 120, is placed less water than will be ultimately needed to dilute the
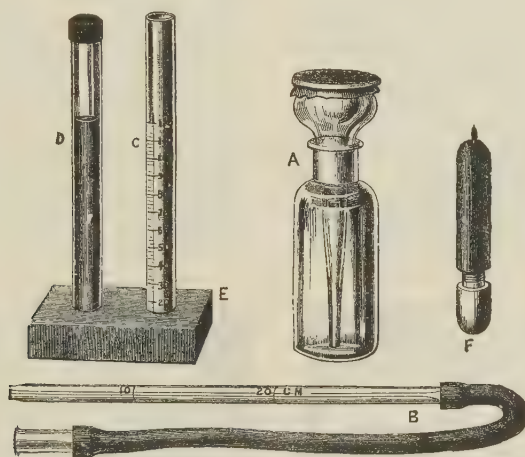
FIG. 52.

blood to the required tint. It contains 2 c.c. when filled up to the mark 100.

The finger is pricked with the lancet and blood is sucked up into the capillary pipette a little beyond the mark 20 (= 20 cmm. or 0·02 c.c.). The point of the capillary is wiped clean and the pipette is dabbed on the back of the hand until the blood stands at the mark. The blood is blown to the bottom of the tube below the water ; the pipette is rinsed out with the water above it. The solution is carefully diluted and mixed with water saturated with coal gas and its tint is compared with that of the standard. Two readings should be taken : (1) when the colours are the same, (2) when the colour is appreciably lighter. The mean of these is taken. The scale gives percentages of hæmoglobin.

*

(4) *Sahli's Method.*

In principle and practice this method is the same as Haldane's method, but the standard is a small tube of acid hæmatin. 20 cmm. of blood are put into ·1N hydrochloric acid in the companion tube. Conversion to acid hæmatin takes place and the solution is diluted until it matches the standard.

In many respects this method is preferable to the previous ones.

B. By Determination of the Oxygen Capacity.

The determination of the oxygen capacity of blood is described on p. 491.

The amount of hæmoglobin can be calculated from the following data :—
1 c.c. of normal blood combines with ·185 c.c. of O_2 at 0° and 760 mm.
1 gm. of hæmoglobin combines with 1·34 c.c. of O_2 at 0° and 760 mm. from which it is found that
1 c.c. normal blood contains ·138 gm. of hæmoglobin.

Normal blood thus combines with 18·5 per cent. of oxygen at 0° and 760 mm. and contains 13·8 per cent. of hæmoglobin.   If the hæmoglobin is expressed as 100, the oxygen capacity value must be multiplied by $\dfrac{100}{18\cdot5}$ or 5·4.

CRYSTALS OF OXYHÆMOGLOBIN.

Crystals of oxyhæmoglobin can be prepared from the blood of different animals.   The ease with which they are obtained differs very considerably and numerous methods have been tried.   Generally, only sufficient crystals for microscopic examination can be prepared ; the preparation is difficult and
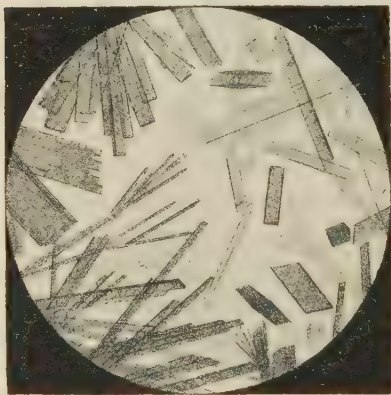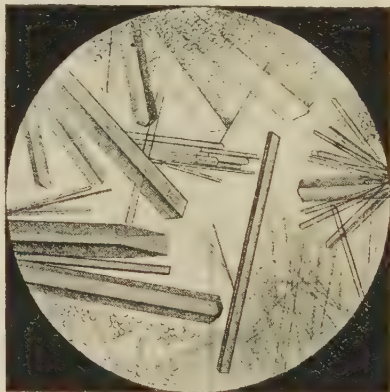
FIG. 53.—Human.   FIG. 54.—Cat.
(After Funke.)

frequently unsuccessful.   Larger quantities of crystals can be prepared most easily from the blood of the horse.

The crystals of oxyhæmoglobin obtained from the blood of different animals are generally microscopic, seldom exceeding 5 mm. in length and of a yellow-red colour.   They vary in shape.   Figs. 53 to 56 show some of the forms of crystalline oxyhæmoglobin.   Those from guinea-pig's and rat's blood are generally tetrahedra or octahedra, those from squirrel's blood are

six-sided plates, those from goose's blood are rhombic plates, those from dog's and horse's blood are usually four-sided prisms.

They generally contain water of crystallisation—an amount of 14·35 per cent. has been evolved on heating crystals dried *in vacuo* to 116°. The crystals, if carefully dried at low temperatures, can be heated to 100° without decomposition, but decomposition occurs if they are moist, as shown by the change in colour.

Oxyhæmoglobin crystals dissolve more or less readily in water according to the blood from which they have been prepared; they also dissolve in dilute alcohol, but are insoluble in ether, chloroform, benzene and most other organic solvents.

Dilute aqueous solutions are more stable than concentrated ones and

FIG. 55.—Squirrel.   FIG. 56.—Guinea-pig.
(After Funke.)

solutions containing a few drops of alkali carbonate are more stable than neutral solutions.

Solutions of oxyhæmoglobin show the colour reactions for proteins but the reactions are more or less masked by the pigment. They coagulate on heating and are precipitated by alcohol and strong acids. Decomposition into hæmatin and globin occurs. They are not precipitated by lead acetate, but they are precipitated by solutions of the alkaloidal reagents in acid solution.

Solutions of oxyhæmoglobin are dextrorotatory (+ 10° for a 1·2 per cent. solution).

TESTS FOR BLOOD STAINS.

(1) *Peroxidase Reactions.*

The stain is dissolved and tested with guaiacum, benzidine and other reagents. The solution should be boiled, so as to exclude the presence of peroxidase, and it should be remembered that other compounds such as halogens give the reaction.

This test serves chiefly as a guide.

(2) *Spectroscopic Examination.*

The absorption spectra of hæmoglobin and its derivatives afford the best means of detecting blood in the form of spots, stains, etc.

The spot is scraped off the material—after its position has been carefully noted. The material, or, if on cloth, a piece of the cloth, is soaked in a small quantity of water, or ·9 per cent. sodium chloride. If the solution is coloured it is examined with a spectroscope, small amounts with a micro-spectroscope, i.e. a spectroscope attached to a microscope.

If the solution is not coloured or coloured too little to show absorption bands, the material is warmed with a little caustic soda solution. Hæmatin is formed and shown to be present by conversion by reduction with ammonium sulphide to hæmochromogen. Both absorption bands may be seen, but generally only one; this band is visible even in very dilute solution.

*   (3) *Teichmann's Test. Formation of Hæmin.*

This test depends upon the formation of hæmin crystals and is carried out in the same way as with a small drop of blood. The blood stain is scraped up and treated with acetic acid as on p. 481.

Stains on iron work are said not to give the crystals.

A stain on cloth which cannot be scraped off is dissolved in glacial acetic acid. If the stain be old, it is necessary to add a crystal of sodium chloride, as the chlorides may have been dissolved out.

Dilute solutions of blood are precipitated by acidifying with acetic acid and adding tannic acid (freshly prepared). The dried precipitate is heated on a slide with a trace of sodium chloride and glacial acetic acid.

Various modifications have been suggested for this method.[1]

THE GASES OF THE BLOOD.

Hæmoglobin as Carrier of Oxygen.

The solubility of oxygen at N.T.P. in water is about ·4 c.c. in 100 c.c.; in blood it is about ·7 c.c. in 100 c.c., excluding the amount in combination with hæmoglobin. This quantity of oxygen in the blood does not suffice to supply the needs of the body for oxygen. Hæmoglobin has the function of being a special solvent for oxygen and of acting as the carrier of oxygen to the tissues. 1 gm. of hæmoglobin combines with 1·34 c.c. of oxygen at N.T.P. This quantity of oxygen is so definite that it is generally believed that the oxygen is in actual chemical combination, but it is thought also that it is simply in solution in the hæmoglobin (by adsorption).

[1] See Biochem. J., Vols. VII. and VIII.

Hæmoglobin readily combines with oxygen and readily gives it up again, especially under the conditions in blood where the temperature is relatively high (37°), salts and acids, especially carbonic acid, are present. Under these conditions the dissociation of oxygen from the hæmoglobin is as rapid as the association of oxygen by the hæmoglobin; they occur at about the same rate, in less than one second, i.e. in about the same time as the flow of blood through the capillaries. Pure hæmoglobin solutions behave differently, the association is rapid, the dissociation is slow in comparison.

The association and dissociation of the hæmoglobin follow the same laws that hold for the partial pressure of gases, whether they are in a gaseous state, or dissolved in a liquid in contact with gas. Transference takes place from the region where the partial pressure (or tension if dissolved), or relative amount, of the gas is high to the region where the partial pressure, or the relative amount, of the gas is low. Association occurs in the lungs where the partial pressure of oxygen in the alveolar air is high; dissociation occurs in the tissues where the partial pressure, or tension, of the oxygen in the hæmoglobin is higher than it is in the tissues.

Determinations have been made to ascertain the relative amounts of hæmoglobin and oxyhæmoglobin which are formed when a hæmoglobin (or oxyhæmoglobin) solution is exposed in a closed vessel to different partial pressures of oxygen. These varying partial pressures of oxygen are prepared by mixing oxygen, or air, with nitrogen in the required proportions in the vessels. Small volumes of blood are introduced and exposed to the large volumes of the known gaseous mixtures at a definite temperature until equilibrium is reached. The amount of oxygen in the sample of blood is then determined by the ferricyanide method (p. 493) and expressed as a percentage of the total oxygen capacity of the blood. The following table is typical of the results obtained; the percentage of hæmoglobin is found by deducting the percentage of oxyhæmoglobin from 100.

Partial pressure of oxygen in mm.	0	5	10	20	50	100
Percentage of oxyhæmoglobin	0	37	55	72	87	94
Percentage of hæmoglobin .	100	63	45	28	13	6

The results can be expressed in the form of a curve, the dissociation curve.

The hæmoglobin is almost completely associated with oxygen at 100 mm. ; at atmospheric oxygen pressure, $\frac{760}{5}$ or 150 mm. the association would be complete. The partial pressure of the oxygen in the lung is about 100 mm., or about 13·2 per cent. of the atmospheric pressure, so that in the lungs the hæmoglobin becomes nearly saturated with oxygen ; at 10 mm. partial pressure about equal parts of hæmoglobin and oxyhæmoglobin are present. Over 30 per cent. of oxyhæmoglobin is still present at 5 mm. partial pressure.

The hæmoglobin in the body is never completely saturated with oxygen, except when it is in contact with the air of the lungs. A sample of blood whether taken from a vein, or artery, always contains a mixture of oxyhæmoglobin and hæmoglobin.

The Blood as Carrier of Carbon Dioxide.

The solubility of carbon dioxide in water at N.T.P. is about 100 c.c. in 100 c.c. The solubility in blood is about the same figure, but an additional amount dissolves in the alkali of the medium as carbonate and bicarbonate, and the proteins of the blood also form an unstable combination with carbon dioxide. Buckmaster[1] found that the amount of carbon dioxide absorbed by blood rises and falls with the amount of hæmoglobin, and concluded that hæmoglobin was capable of absorbing considerable quantities of carbon dioxide at pressures between 70 and 760 mm. Consequently, hæmoglobin has also the function of carrier of carbon dioxide.

The amount of carbon dioxide in solution in the blood does not approach saturation of the liquid with the gas. 100 volumes of venous blood contain about 46 volumes of carbon dioxide. Most of the carbon dioxide is in combination as carbonates, only about 5 per cent. being actually in solution. This has been determined by a similar method to that used in ascertaining the saturation of hæmoglobin—by exposing blood to different amounts of carbon dioxide and analysing the gas before and after the experiment. Blood absorbs carbon dioxide at a partial pressure of carbon dioxide higher than 5 mm. and gives it up at a partial pressure lower than 5 mm.

The carbon dioxide exchange in the animal body is in the converse order to that of the oxygen.

The Blood as a Solvent of Nitrogen.

Water dissolves 2 c.c. of nitrogen at N.T.P. per 100 c.c. A little more than this amount is dissolved by blood, but the amount of

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[1] J. Physiol., 1917, **51**, 164.

nitrogen in water, or in blood, exposed to the atmosphere which contains 4/5 of its volume as nitrogen is $\frac{4}{5} \times 2$ or 1·6 c.c. This amount of nitrogen is always present in blood, whether it be arterial, or venous.

The average content of arterial and venous blood is :—

	Arterial Blood.	Venous Blood.	
Oxygen . . .	20	8-12	
Carbon Dioxide . .	40	46·50	c.c. per 100 c.c.
Nitrogen . . .	1-2	1-2	

The nitrogen is the same in both ; arterial blood contains 8-12 c.c. more oxygen than venous and 6-10 c.c. less carbon dioxide.

THE ESTIMATION OF OXYGEN AND CARBON DIOXIDE IN BLOOD.

The Oxygen Capacity of Blood.

Haldane has shown that when oxyhæmoglobin is treated with potassium ferricyanide the whole of the oxygen of the oxyhæmoglobin is evolved and methæmoglobin is formed. The amount of oxygen in blood can thus be determined.

Circulating blood contains a mixture of oxyhæmoglobin and hæmoglobin. The oxygen given off from such blood is a measure of its oxygen content.

The total amount of oxygen which is obtainable from blood which has been exposed to the air so that it contains only oxyhæmoglobin is the oxygen capacity of the blood.

The oxygen capacity depends on the total amount of hæmoglobin in the blood and thus indirectly it will give the hæmoglobin content (p. 486).

Carbon dioxide is liberated from carbonates by the action of acids. The amount in blood can therefore be estimated in this way.

(1) *Approximate Method.*

Haldane[1] originally estimated the oxygen and oxygen capacity in an apparatus which was almost identical with the Dupré apparatus for estimating urea (p. 129). A very fair estimation can be performed in such an apparatus. The burette should be of 20 c.c. and the graduations should be to 0·05 c.c.

20 c.c. of (defibrinated) blood are measured out with a pipette and introduced into the bottle of about 120 c.c. capacity. The last drops of blood must not be blown out, but are expelled by closing the top of the pipette with the finger and warming the bulb with the

hand.   30 c.c. of dilute ammonia solution (4 c.c. of ammonia of sp. gr. ·880 in 1000 c.c. of distilled water) are added and mixed with the blood.   The ammonia prevents the evolution of carbonic acid, whilst the water lakes the corpuscles.   The solution should be quite transparent. If the laking be not complete, more ammonia must be added.

4 c.c. of saturated potassium ferricyanide solution are put in the small tube, which should be slightly longer than the width of the bottle, and it is placed upright in the large bottle without spilling.   The bottle is closed with a rubber stopper through which a glass T-tube passes ; this is connected at one end with the burette in a cylinder of water by india-rubber tubing and closed at the other end with a clip.   The bottle is put in a vessel of water of the same temperature as that of the room.   By opening the clip, the level of the water in the burette is brought nearly to the top and to the same height as the water in the cylinder.   The clip is closed and the height in the burette is read off.

The bottle is tilted so as to upset the ferricyanide solution and *shaken gently* as long as gas is evolved, the little tube being repeatedly emptied so that all the oxygen is given off.   When the oxygen ceases to be evolved, the bottle is replaced in the water and cold or hot water is added to it until it attains the same temperature as at the commencement.   The burette is adjusted so that the water has the same level inside and outside and the volume of the gas is read off.   The difference in readings gives the amount of oxygen evolved from 20 c.c. of blood.

This volume should be reduced to 0° and 760 mm. and a correction should be made for the pipette which delivers only about 19·6 c.c. of blood instead of 20 c.c. of blood.

* The oxygen capacity is determined in the same way, but 25-30 c.c. of the blood are first saturated with oxygen by spreading over the surface of a large flask without producing a froth.

3·7 c.c. are given off by 20 c.c. of normal blood containing 13·8 per cent. of hæmoglobin.

Estimation of Carbon Dioxide in Blood.

A rough estimation of the carbon dioxide in blood can be made in the same apparatus.   The oxygen is first estimated.   The carbon dioxide is liberated by disconnecting the apparatus, putting 5 c.c. of 20 per cent. tartaric acid in the small tube, again connecting the various parts and upsetting the tartaric acid into the liquid and measuring the

gas evolved. The gas should be measured at constant pressure and correction made for the solubility of the carbon dioxide in water.

In this estimation it would be preferable to use 50 per cent. glycerol solution, as suggested by Edkins for gas analysis (p. 513), which does not appreciably dissolve carbon dioxide.

(2) *Exact Method.*

Very accurate results are given by the apparatus devised by Haldane in 1920. The essential feature of this method is the provision of a special compensating device to allow for changes in temperature and barometric pressure during the course of the experiment. The apparatus (Fig. 57) consists of two small round flasks A and B which can be im-
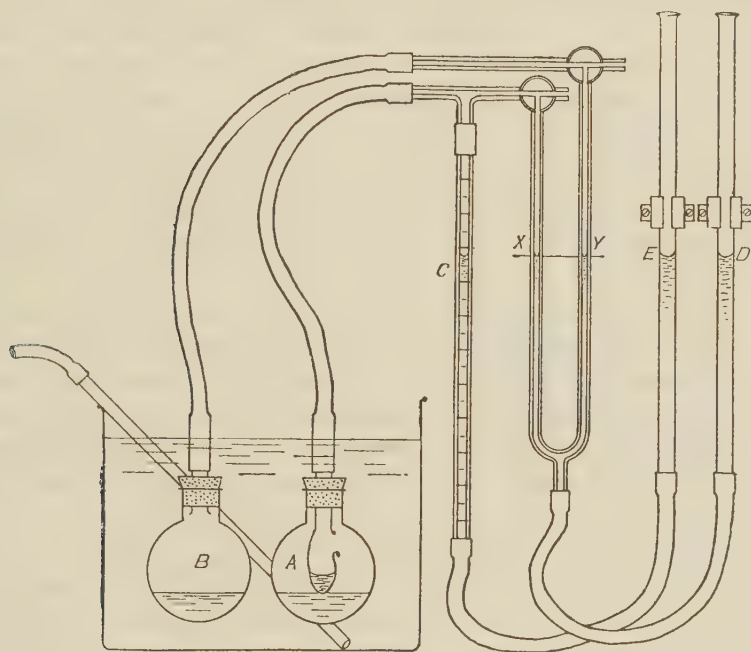
FIG. 57.
By kind permission from Douglas and Priestley's " Human Physiology."
Clarendon Press, Oxford, 1924.

mersed in a bath of water so as to be at the same temperature. Flask B acts as compensator. It is filled with 2 c.c. of water and is fitted with cork and glass tube, and is connected by narrow bore rubber tubing to a glass T tube with 3-way tap, which in turn is connected to one limb Y of a U-tube gauge. Flask A is fitted with cork and glass tube. The glass tube extends into the flask and has a hole in its side which allows the end portion to be filled with ferricyanide, or tartaric acid, solution. It is connected by rubber tubing to a T piece, one limb of which is

connected to a gas burette of 1 c.c. capacity and graduated to 0·01 c.c. (reading to 0·001 c.c.) with levelling tube E, and the other limb through a 3-way tap to the other limb X of the U gauge. The U gauge is, connected to a levelling tube D. The gas burette, U gauge, and levelling tubes are filled with sufficient water, preferably coloured by eosin and containing a trace of bile salt to ensure free movement of the water in the narrow tubes. Any variation in temperature is shown by flask B, and movement in the link Y of the U tube is compensated for by moving the levelling tube D. The same alteration is made mechanically to limb X and flask A and its connections. The water in the bath is stirred by blowing air through it.

The estimation of oxygen is carried out as follows :—

2 c.c. of 1 per cent. anhydrous sodium carbonate solution is placed in flask A to retain the carbon dioxide of the blood. A minute quantity of saponin is added to it to lake the blood. 2 c.c. of defibrinated blood, or blood treated with 0·1 per cent. of sodium oxalate to prevent clotting, exposed to the air to be completely oxygenated, are run in slowly from a pipette under the carbonate solution. The pipette should be so graduated that the last quantity of blood in it is not delivered. The blood and carbonate are mixed by gently shaking : if the blood be not laked in a minute or two, a trace more saponin must be added.

0·25 c.c. potassium ferricyanide solution is placed in the glass tube in the cork through the hole in its side, together with a trace of bile salt to assist in its flowing out. The apparatus is now carefully fitted together with both 3-way taps open to the air, the apparatus is left for 10 minutes to attain the temperature of the bath, the water in the bath being stirred from time to time. The water level in the burette is adjusted to 0·1 c.c. and that in the gauge is adjusted to definite marks. The taps are now closed to the air, but open to the flasks and U-gauge. The water levels are adjusted exactly to their marks and the burette is read. At intervals of 1 minute with stirring of the water in the bath, the levels are again adjusted and the burette read until the reading is constant. The bottle A is grasped by tongs (to avoid heating from the hand), lifted from the water-bath and tilted to upset the ferricyanide solution. The flask is returned to the bath and shaken with a rotary movement, thus avoiding frothing. The oxygen is liberated and collects in the gas burette. At the same time the levelling tube E is adjusted to keep the liquid in the limb at its mark. After 3 minutes' shaking, the levels of the water in the U-gauge are adjusted to the marks and the volume in the burette is read. The reading is repeated after half a minute's further shaking, and if there is alteration further shaking is necessary till the volume remains

constant. The temperature is indicated by a thermometer fixed close to the burette. The volume is reduced to N.T.P., with allowance for tension of aqueous vapour at the temperature indicated, and the result calculated for 100 c.c. blood.

The estimation of carbon dioxide is made in a similar way :—

Instead of carbonate solution 1·5 c.c. of dilute ammonia solution,[1] with a trace of saponin, is placed in the flask. If the blood is not immediately run in, the bottle must be stoppered to prevent absorption of carbon dioxide from the air. 1 c.c. of blood is added, and 0·25 c.c. of potassium ferricyanide and the oxygen is evolved by shaking.

0·25 c.c. of 20 per cent. tartaric acid solution is put in the tube through the small hole, the parts of the apparatus are connected and the estimation is carried out in the same way as for oxygen.

Owing to the effect of the tartaric acid upon the ammonia solution which causes liberation of carbon dioxide from it, if present, or absorption of ammonia vapour, a blank experiment with 1 c.c. boiled distilled water instead of blood should be carried out and the figure so obtained allowed for in the actual estimation.

Before calculating the final result, allowance must be made for carbon dioxide left in the aqueous solution on account of its solubility. The capacity of the bottle (about 20 c.c.) together with that of the glass tubing through the cork (about 0·5 c.c.) must be ascertained by measurement. The volume of this gas space, say, 20·5 c.c. less the liquid 3 c.c. is thus 17·5 c.c. At 13° C. the coefficient of absorption of CO_2 is 1·0 ; above and below 13° it decreases, or increases, by $\frac{1}{40}$ for each degree. If the observed volume at N.T.P. be 46·0 c.c. at 13°, the volume on correction is $46 \times \dfrac{20\cdot5}{17\cdot5}$ c.c. per 100 c.c. blood, or 53·9 c.c., i.e. an increase of 17·2 per cent. $\frac{1}{40}$ of 17·2 per cent., or 0·43, must therefore be added, or subtracted, for each degree,

e.g. at 16° the volume would be $46 + \left(\dfrac{17\cdot2 - 3 \times \cdot43}{100} \times 46 \right)$

at 12° ,, ,, ,, $46 + \left(\dfrac{17\cdot2 + 0\cdot43}{100} \times 46 \right)$.

THE SIMPLE SOLUBLE CONSTITUENTS OF BLOOD.

Blood contains mineral salts, amino acids, creatinine, purines and uric acid, glucose and other compounds in solution. Their amount

[1] Prepared from ·880 ammonia shaken with caustic lime to remove carbon dioxide and boiled distilled water. 4 c.c. per litre of water.

varies within narrow limits and requires estimation in complex meta-bolism studies.   The most important of these constituents is glucose.

Glucose is normally present in blood to the extent of about 0·1 per cent.   In glycosuria and diabetes the amount rises above this figure to about 0·2 to 0·3 per cent.   Special treatment by feeding, or by insulin, may reduce the excess to normal.   The progress of the treatment can be followed by estimation of the glucose content of the blood.

Estimation of Glucose in Blood.

Numerous methods have been devised for the rapid estimation of glucose in small quantities of 0·1 to 1 c.c. of blood.   One of the simplest and easiest to carry out is that of Maclean.[1]   That of Shaffer and Hartmann [2] is essentially the same.   In all methods, protein must be first removed.

The determination of the sugar in the filtrate in Maclean's method is effected by reduction of an alkaline copper solution and the estima-tion of cuprous oxide by means of its oxidation by iodine liberated from iodate and iodide.   Excess is added and is estimated by thio-sulphate.

The reactions of the process are :—

$$2CuSO_4 \rightarrow Cu_2O$$
$$Cu_2O + I_2 + H_2O = 2CuO + 2HI.$$
$$KIO_3 + 5KI + 3H_2SO_4 = 3I_2 + 3K_2SO_4 + 3H_2O$$
$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6.$$
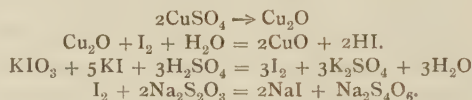
In carrying out the method the chief source of error is in the reduction of the alkalin ecopper solution.   This must be accomplished under a standard set of conditions.   The procedure may be divided into four stages :—

(1) Drawing and measurement of the blood.

(2) Removal of protein from the blood.

(3) Standardisation of the conditions of reduction, and of the reagent.

(4) Estimation of the glucose.

1. *Drawing and Measurement of the Blood.*

Blood is drawn from the finger, or ear.   The upper part of the finger is bound round with rubber tubing to produce congestion and the finger is pricked with a needle just above the root of the nail.   The point of a *perfectly clean* [3] special 0·2 c.c. pipette is placed at the side of

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[1] Biochem. J., 1919, **13**, 135.   [2] J. Biol. Chem., 1920-21, **45**, 365.

[3] The pipette is cleaned by drawing into it a hot mixture of bichromate and sulphuric acid.   It is washed out with water, alcohol and ether and dried by blowing a current of air through it.   It is best to keep the pipette in the bichromate sulphuric acid mixture, and to wash and dry it just before use.

the drop. Blood flows into the pipette, and the pipette becomes filled, if it is held horizontally so that gravity assists the flow. Blood on the point is wiped away, and if too much has entered the pipette the excess is got rid of by gently tapping the pipette against the thumb nail. The finger may be pricked again, if insufficient blood has been secured. Coagulation does not occur after some practice with the method, but it may be prevented by dusting the part of the finger with very finely powdered potassium oxalate.

(2) *Preparation of Protein-free Filtrate.*

The 0·2 c.c. blood is put into exactly 23·8 c.c. of acid sodium sulphate[1] solution contained in a 100 c.c. conical flask of heat resisting glass. The pipette is washed out by sucking in and blowing out the solution. A rubber stopper carrying a glass tube drawn out to a capillary point, the point projecting into the flask, is inserted, and the solution. is heated just to boiling as indicated by the appearance of a few bubbles. The object of the capillary is to prevent escape of steam. The flask is allowed to cool for a minute, the stopper loosened and withdrawn sufficiently to allow the introduction of the point of a pipette so as to add 1 c.c. of dialysed iron.[2] The stopper is replaced and the contents well shaken, any drops on the sides of the flask being mixed with the bulk of the liquid. The contents are cooled under running tap water and filtered through a 9 cm. No. 1 Whatman filter paper into a dry cylinder. The whole filtrate must be collected, as 20 c.c. are required for the glucose estimation.

(3) *Standardisation and Heating of Reagents.*

The most important part of the whole procedure is in the heating of the alkaline copper reagent. The amount of reduction depends upon the rate and time of the heating. This is easily controlled and kept constant by the introduction of a manometer in the gas supply (Fig. 58). Such a manometer is easily made from bent glass tubing and a T-piece, connected by rubber tubing. The manometer is filled with coloured water.

The adjustment of the heating is made by trial with 22 c.c. of acid sodium sulphate solution in a 100 c.c. conical flask. It is placed upon a gauze on a stand from $\frac{1}{4}$ to $\frac{1}{2}''$ above a burner which must be protected

[1] 0·1 c.c. pure acetic acid, redistilled over potassium permanganate, is added to 100 c.c. of 15 per cent. sodium sulphate. This does not keep and must be made from stock sodium sulphate solution.

For one or two estimations, 1 drop of 50 per cent. acetic acid may be added to 23·8 c.c. of stock sodium sulphate solution.

[2] The dialysed iron must be free from chlorides.

by a chimney.   The gas is turned on full and reduced by means of a
screw clip on the rubber tubing.   The solution is heated and must be
raised to vigorous boiling in about 1 min. 40 sec. from a starting
temperature of 20°.
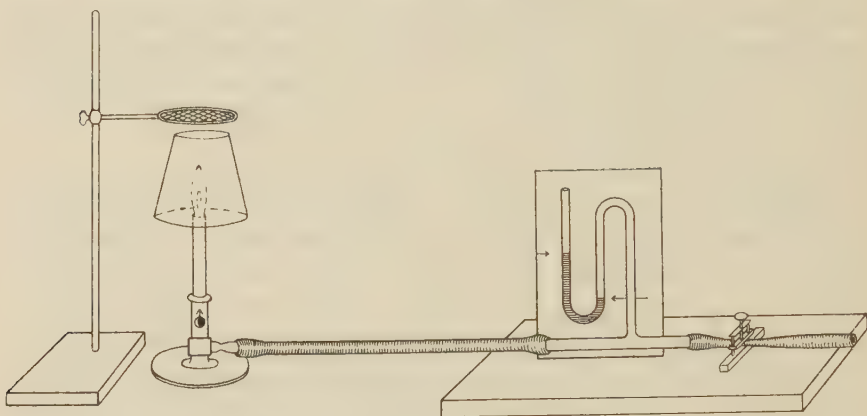
Fig. 58.
From Biochemical Journal, 1919, **13**, 141.

Having found the position of the screw clip to effect this, a further
trial is made with 20 c.c. acid sodium sulphate solution and 2 c.c. of the
copper solution.[1]   The manometer will now indicate the gas pressure to
produce boiling in 1 min. 40 secs.   The position of the liquid in the
limbs is carefully marked.   Also, the position of the air regulator on the
burner must be marked with a file, or better it is kept in the same
position by introducing a wedge.

The copper solution can now be standardised.   20 c.c. of acid sodium
sulphate and 2 c.c. of copper solution are placed in the flask.   The
flask is raised to the boil, and kept briskly boiling for exactly six
minutes.   It is removed and cooled in a basin of cold water.   To the
cooled solution, 2 c.c. of 25 per cent. sulphuric acid[2] are added and the

[1] *Copper Solution.*—This consists of —

Potassium bicarbonate	12 grams.
Potassium carbonate (anhydrous)	8 ,,
Copper sulphate (crystals)	0·35 ,,
Potassium iodate	0·05 ,,
Potassium iodide	0·50 ,,
Water	to 100 c.c.

it is prepared by dissolving the bicarbonate in 60-70 c.c. water with gentle heating and then
adding the carbonate.   The copper, dissolved separately in a few c.c. of water, is added be-
fore complete solution occurs.   After effervescence ceases, complete solution is brought
about.   The iodate and iodide are then added, the solution shaken and filtered through a
No. 1 Whatman paper.   The solution should stand for a few days before use.   It keeps
indefinitely.

[2] 25 c.c. concentrated H_2SO_4 to 75 c.c. H_2O.

flask gently shaken till effervescence ceases.   It is allowed to stand with
an occasional rotary shake for 1 minute.   Titration is then carried out
with N/400 thiosulphate [1] till the yellow colour almost disappears.   Two
drops of soluble starch solution [2] are added and titration continued till
the solution is colourless.   The end point is quite sharp.

This titration gives the total iodine in the solution.

(4) *Estimation of the Glucose.*

20 c.c. of blood filtrate (10 c.c. of that of diabetic blood + 10 c.c.
acid sodium sulphate solution) are put into the conical flask, raised to
the boil in 1′ 40″, boiled briskly for 6 minutes, cooled, treated with
2 c.c. of 25 per cent. sulphuric acid and after standing titrated with
N/400 thiosulphate.

The difference between this reading and the above gives the amount
of sugar as per table,

e.g. standard, 11·5 c.c. ; blood filtrate, 9·8 c.c.

Difference = 1·7 c.c. percentage of glucose in blood = 0·093.

If less blood filtrate than 20 c.c. has been used, a correction is made

by multiplying the percentage by $\dfrac{20}{x}$, where x is the number of c.c.

used.

C.c. N/400 Sodium Thiosulphate = Percentage of Glucose with 20 c.c. Blood
Filtrate from 0·2 c.c. Blood (Maclean).[3]

c.c. N/400 Thiosulphate.	Percentage of Glucose.	c.c. N/400 Thiosulphate.	Percentage of Glucose.	c.c. N/400 Thiosulphate.	Percentage of Glucose.
0·12	0·018	2·22	0·118	4·24	0·218
0·25	0·025	2·35	0·125	4·37	0·225
0·38	0·031	2·14	0·131	4·49	0·231
0·50	0·037	2·61	0·137	4·62	0·237
0·62	0·043	2·74	0·143	4·74	0·243
0·73	0·050	2·86	0·150	4·87	0·250
0·86	0·056	2·99	0·156	4·99	0·256
0·99	0·062	3·11	0·162	5·12	0·262
1·13	0·068	3·24	0·168	5·24	0·268
1·26	0·075	3·36	0·175	5·37	0·275
1·39	0·081	3·49	0·181	5·49	0·281
1·53	0·086	3·61	0·187	5·62	0·287
1·67	0·093	3·74	0·193	5·74	0·293
1·80	0·100	3·87	0·200	5·87	0·300
1·94	0·105	3·99	0·206	5·99	0·306
2·07	0·112	4·12	0·212	6·12	0·312

[1] This must be freshly prepared from ·1N thiosulphate by diluting 5 c.c. to 200 c.c.
·1N thiosulphate (24·8 gm. $Na_2S_2O_3 . 5H_2O$ per litre) should be kept in an amber bottle away
from the light.

[2] 1 per cent.

[3] "Modern Methods in the Diagnosis and Treatment of Glycosuria and Diabetes."
Constable & Co., Ltd., 1924.

CHAPTER L.

METABOLISM.

THE complete series of changes which the various chemical compounds undergo in their synthesis and degradation in the body is still unknown, so that it is not possible to draw up a full and proper balance-sheet of every detail. But a balance-sheet representing the intake and the output can be more or less satisfactorily drawn up. The amount of protein, fat and carbohydrate, mineral matter, and other constituents in the food of animals and the oxygen entering by the lungs as well as the amount of carbon dioxide in the expired air, ammonia, urea, etc., in their excreta, can be determined. A known weight of carbohydrate, or fat, eaten by an animal is represented by the corresponding weight of carbon dioxide evolved through the lungs, unless some is retained in the body, in which case the body weight of the animal will increase. Similarly, a known weight of protein, or nitrogenous food, will be represented by the corresponding weight of nitrogen in the urine. The actual amount of each constituent ingested by an animal is the amount consumed less the amount contained in the fæces, which consists of undigested food, i.e. food which has not entered the circulation. When the intake and the output of the constituents is the same, then the animal is said to be in equilibrium; when less is put out than is taken in, then there is retention, or assimilation, and increase of body weight and a positive balance; when the output is greater, then there is loss of weight and a condition of negative balance. The whole series of changes is generally referred to as metabolism. Those changes leading to building up of tissue are called anabolic, and those leading to breaking down catabolic.

The foodstuffs in their catabolism in the body are oxidised and accompanied by an evolution of heat. The living organism conforms to the physical laws of conservation of energy. The heat evolved serves to maintain the body temperature and for the various muscular movements. The total heat evolved by the body corresponds to the heat value of the food. Each compound has a certain caloric value and

can evolve on complete combustion a definite amount of heat.   The physiological heat value of

1 gm. of protein is	4·1 large calories[1];	1 oz. = 116 cal.
1 gm. of fat is	9·3 „ „ ;	1 „ = 263 „
1 gm. of carbohydrate is	4·1 „ „ ;	1 „ = 116 „

The Energy Metabolism.

The energy metabolism is represented on the one side by the caloric value of the food in terms of fat, protein, carbohydrate, and on the other side by the amount of heat given off.   A man, or animal, is placed in a special form of calorimeter through which air of a constant temperature can be passed and the heat evolved measured.   The experiments are complicated and difficult to perform.   Reference must be made to the special books upon the subject for the details.

In order to produce the energy balance-sheet, the amount of energy expended by the body under different conditions, such as sitting, running, heavy muscular work is determined.   From the energy output the amount of food needed to compensate can then be calculated.

As the energy output varies with the occupation of the individual, the energy metabolism is divided into 2 parts :—

(1) Basal metabolism—the amount to maintain warmth and to give energy for respiratory movements, heart contractions, etc.

(2) Work metabolism—the amount in addition to the basal metabolism to supply energy for body movement.

These amounts are determined by placing the individual in a calorimeter in a fasting condition (12 hours after the last meal) at rest, i.e. lying quietly on a bed for basal metabolism, sitting, standing, or riding a fixed bicycle against a resistance for work metabolism.   Each operation is carried out only for a short time and the quantity calculated for 24 hours.

The basal metabolism has been found to vary with the individual and with his surface area.   It is greater per kilo. the smaller the animal. This result is due to the chief energy output being required to maintain body temperature and a smaller animal having relatively a larger surface. Basal metabolism is a function of the body surface.   The surface is usually reckoned from Du Bois' formula :

$$S = ·007184 \times H^{0·725} \times W^{0·425}$$

where H is the height in centimetres, W the weight in kilograms, and S the surface in square metres.

[1] A large calorie is the heat required to raise 1000 gm. of water from 15°-16° (= 1000 small calories).

The basal metabolism of man is about 40 cals. per sq. m. per hour.

The energy output varies also with size and age. The average woman and child have a lower energy metabolism than man. Their energy requirements are generally calculated from those of an adult man using Lusk's coefficients :—

man 1   boys over fourteen 1   children, ten to fourteen 0·83
woman 0·83 girls over fourteen 0·83   ,,   six to ten   0·7
                                            under six   0·5.

A man of the average weight of 70 kilos (11 stone) performing light muscular work, *under starvation conditions*, loses 2240 large Calories a day. To counterbalance this loss, and making allowance for non-utilisation in the body of some of the food (10 per cent.), a diet yielding from 2500-2700 Calories is required. For other conditions, the standards usually adopted are those of Atwater, namely,

3000 Calories for light   muscular work
3500   ,,   medium   ,,   ,,
4500   ,,   heavy   ,,   ,,

The main portion of the caloric value of the food is supplied by the carbohydrate and fat of the diet. The quantities of these foodstuffs are increased, or diminished, so as to produce the final necessary caloric value. The supply of protein need only be varied very slightly, 90-110 gm., 125 gm., 150 gm., for light, medium and heavy work respectively.

The chief question in compounding the average diet is in respect of the amount of protein. Although the average is taken at 90-100 gm. protein, it has been shown by Chittenden that 60-70 grams of protein will suffice and Hindhede has found that an adult man can be maintained upon 30-40 grams of purely vegetable protein daily. The value of a protein in nutrition depends upon the amino acids present (p. 367). Animal proteins, containing all the amino acids, are good proteins. Vegetable proteins which may lack certain amino acids, e.g. zein, or contain very little of an amino acid, e.g. wheat gliadin, are poor proteins.

The following diets have been accepted as standards :—

A. The old standard :

carbohydrate 500 gm. giving a Caloric value of 2050
fat   60 ,,   ,,   ,,   ,,   558
protein   100 ,,   ,,   ,,   ,,   410
                                         ———
                           Total   3018

B. The War diet standard of the Royal Society Food Committee :

carbohydrate 550 gm. giving a Caloric value of 2200
fat 90 ,, ,, ,, ,, 810
protein 70 ,, ,, ,, ,, 280
_____
Total 3290

C. Average diet under normal conditions (Royal Society Food Committee) :

carbohydrate 500 gm. giving a Caloric value of 2050
fat 100 ,, ,, ,, ,, 930
protein 100 ,, ,, ,, ,, 410
_____
Total 3390

It should be noted that these figures represent dry weight, and require recalculation in terms of the actual foodstuffs (see analyses, p. 506).

The great difficulty in fixing the quantity of protein arises from the varied proportion of the amino acids. About half the total protein is generally supplied by animal tissue. If the ratios of the food quantities be calculated from the above figures, it is seen that

protein is $1/7$, fat is $1/7$ and carbohydrate is $5/7$

or approx. ,, $1/6$, ,, $1/6$,, ,, $2/3$.

The ratio of the food constituents of milk is :

protein is $1/3.5$, fat is $1/3.2$ and carbohydrate is $1/2.4$

or approx. ,, $\frac{1}{4}$,, $\frac{1}{4}$,, ,, $\frac{1}{2}$

In changing from milk to a mixed diet, the protein and fat are reduced and the carbohydrate increased. In judging a diet, it is of importance to consider the ratios (dry weight) of the several constituents. These ratios should be retained in raising the quantity of food to produce a higher caloric value, rather than simply increasing the amount of carbohydrate or fat.

The Carbon Metabolism.

The carbon metabolism is ascertained from the analysis of the inspired and expired air over a period of several hours. The animal, or man, is placed in a suitable chamber, the volume of gas which enters and leaves is measured and samples of each are analysed.

The subject may be in fasting condition, fed upon any desired food, or mixture of foods, or be in a resting or active state. One side of the

balance-sheet is given by the analyses of foods. The carbon dioxide produced is derived from the oxidation of the three classes of foodstuffs. The amount of protein oxidised is given by the nitrogen estimation of the urine : 1 gm. of nitrogen corresponds to 8·4 grams of oxygen and 9·35 gm. of carbon dioxide. If these amounts be deducted, the remainder is from the oxidation of fat and carbohydrate.

According to the class of food oxidised in the body, a different volume of oxygen is required, thus :—

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O.$$
Glucose.

$$C_{54}H_{104}O_6 + 77O_2 = 54CO_2 + 52H_2O.$$
Fat.
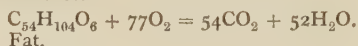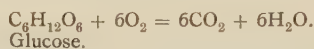
The ratio of $\dfrac{\text{volume of } CO_2}{\text{volume of } O_2}$ is thus $\dfrac{6}{6}$, or 1, in the case of glucose, and $\dfrac{77}{54}$, or 0·7, in the case of fat. In the case of protein it is 0·81. This ratio is termed the respiratory quotient. On a mixed diet, it is between 0·7 and 1, usually about 0·85. If it is near 1, a greater proportion of carbohydrate is being oxidised, and near 0·7, a greater proportion of fat. If carbohydrate is being converted into fat, more carbon dioxide without a corresponding absorption of oxygen occurs ; the respiratory quotient may then rise above 1. Respiratory quotients below 0·7 would indicate a conversion of fat into carbohydrate.

The respiratory exchange leads also to the energy output. The calorie equivalent per litre of oxygen absorbed has been calculated by Zuntz and Schumburg. They give the following table for different values of the respiratory quotient :—

R.Q.	Calories per litre of O_2 Absorbed.	R.Q.	Calories per litre of O_2 Absorbed.	R.Q.	Calories per litre of O_2 Absorbed.
0·70	4·686	0·80	4·801	0·90	4·924
0·71	4·690	0·81	4·813	0·91	4·936
0·72	4·702	0·82	4·825	0·92	4·948
0·73	4·714	0·83	4·838	0·93	4·960
0·74	4·727	0·84	4·850	0·94	4·973
0·75	4·739	0·85	4·863	0·95	4·985
0·76	4·752	0·86	4·875	0·96	4·997
0·77	4·764	0·87	4·887	0·97	5·010
0·78	4·776	0·88	4·900	0·98	5·022
0·79	4·789	0·89	4·912	0·99	5·034
—	—	—	—	1·00	5·047

The Nitrogen Metabolism.

The total nitrogen metabolism is ascertained by the analysis of the nitrogen in the food and in the excreta.

The total nitrogen value is the sum of the amounts of nitrogen in the various compounds present in the food and in the excreta. The protein of the food is really represented by the ammonia and urea in the urine. Purines, creatine, etc., can be specially investigated.

The Metabolism of Inorganic Compounds.

The metabolism of the inorganic compounds is determined by the analysis of sulphates, phosphates, sodium, potassium, etc., in the food and the excreta.

The total nitrogen metabolism, the metabolism of the individual nitrogenous compounds and of the inorganic compounds, and the carbon metabolism can be easily determined and a great deal of information can be drawn from analyses of urine and expired air.

CHAPTER LI.

COMPOSITION OF FOODSTUFFS.

THE principal constituents of all animal and vegetable tissues are water, proteins, carbohydrates, fats, and mineral salts. Various other substances are also present in small amounts, such as amino acids, purines, creatine, lecithine, cholesterol, etc. They are included in the protein, or fat.

Animal tissues consist mainly of protein with fat and only traces of carbohydrates.

In vegetable tissues the amount of carbohydrate preponderates, and the amount of protein is small. Protein is present in greatest amount in the dried legumes, or pulses. Fruits and green leaves consist chiefly of water. Nuts are composed most largely of fat and protein.

The following table shows the percentage amounts of these constituents in the commoner foodstuffs :—[1]

	Water.	Mineral Matter.	Protein.	Fat.	Carbo-hydrate.	Cellulose or Fibre.
Lean meat . .	77	1·3	21	1·5	0·3	—
Cod, white fish .	80·7	1·1	18	0·2	—	—
Herring, mackerel	68·8	1·2	19	11	—	—
Eggs . . .	73·7	1·1	12·3	11·3	1·6	—
Cheese (cheddar) .	31·6	4·7	25·7	35·0	3·1	—
Cow's milk . .	87·6	0·7	3·3	3·6	4·8	—
Human milk .	90	0·2	2·0	3·1	5·0	—
Butter, margarine	13·9	0·4	0·4	84·3	—	—
Wheat flour . .	11·3	0·8	10·1	1·6	75·5	0·7
Bread . . .	43·2	1·9	7·0	0·2	47·0	0·5
Potatoes . .	76·2	0·8	1·6	0	21·0	0·4
Cabbage . .	92·6	0·6	1·4	0·1	4·5	0·8
Peas, beans (dry) .	13·8	2·7	20·4	0·6	57·1	5·4
Nuts (average) .	5	1	20	60	10	—
Fruits (average) .	84	0·4	0·5	—	10	4

Vitamins.

Certain foodstuffs contain in minute quantities substances of unknown chemical composition termed accessory food factors, or vitamins. Their presence in the foodstuffs can only be recognised by feeding experiments

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[1] A complete set of analyses is given in " Analyses and Energy Value of Foods," by R. H. A. Plimmer, 1921. H.M. Stationery Office.

of long duration.   Their absence from the food leads to the so-called deficiency diseases.   Three definite and distinct vitamins have been clearly distinguished and the existence of two more is probable.   In the absence of chemical knowledge they are at present distinguished by the letters A, B, C, D, E.   Recently, it has been found that irradiation of cholesterol produces a substance with properties of vitamin-D, and a colour test has been described for vitamin-A.   The exact function that the vitamins play in nutrition is also unknown, but at any rate they are closely connected with health :—

Absence of vitamin-A leads to wasting and xerophthalmia.

Absence of vitamin-B leads to beri-beri.

Absence of vitamin-C leads to scurvy.

Absence of vitamin-D is associated with imperfect ossification of the bones.

Absence of vitamin-E is associated with failure of reproduction.

It is necessary also to consider the effect of a shortage of the vitamins in the diet.   According to the degree of shortage, the actual symptoms of the particular deficiency disease are delayed.   If the shortage is small, the appearance of the typical symptoms may never be observed, but the animal shows symptoms which become chronic Shortage of vitamins A and D, combined with a shortage of vitamin-B, in the diet is associated with rickets.   Shortage of vitamin-B alone is associated with atrophy of the internal secretory glands, atrophy of the muscle and nerve structures of the intestinal wall, leading to stoppage of the gut and heart symptoms.   Shortage of vitamin-C is associated with a sallow, muddy complexion and pseudo-rheumatic pains.   Many cases of ill-defined bad health are greatly benefited by the consumption of foods containing vitamins.   The following tables show the foodstuffs containing, or not containing, vitamins :—

Vitamins A and D.	*Vitamin-B.*	*Vitamin-C.*
Cod-liver oil.	Whole meal flour.	Fresh fruits, especially oranges,
Fish roe.	Whole cereals.	lemons, tomatoes.
Butter.	Nuts.	Fresh green vegetables.
Animal fats.	Potatoes.	Potatoes.
Egg yolk.	Dried legumes.	
Green vegetables.	Yeast and yeast extract (marmite).	

Corresponding Foods not Containing Vitamins.

Vegetable oils.	White flour.	Overcooked vegetables and vege-
Nut butters.	White rice.	tables cooked with soda.
Lard.	Cornflour.	Most tinned fruits and vege-
	Sago, tapioca.	tables.
	Sugar.	

A full account of the connection between vitamins and health is given in " Food and Health." [1]

[1] By R. H. A. Plimmer and V. G. Plimmer, Longmans, Green & Co.

Analysis of Foods.

The analysis of foods is carried out by the usual methods of chemical analysis, after disintegration of the foodstuff by mincing, grinding, or other mechanical means.   It is usually not possible to reduce the foodstuff to an even fineness on account of the difficulty of breaking up the connective tissue of animal material and the fibrous material of plant tissues.   On this account and also owing to the presence of fat, duplicate analyses may not agree as well as would be expected.   Moisture is also lost during the time of preparation and weighing out of the material.

(1) *Estimation of Water and Ash.*

About 1 gm. of material is heated in a small crucible at 100-110° until the weight is constant.   The dried material is incinerated.

The amount of sodium chloride in the ash may be ascertained by dissolving in dilute nitric acid and estimating by Volhard's method (p. 521).   On deducting the chloride, the remainder is "other ash."

(2) *Estimation of Sodium Chloride.*

The estimation of sodium chloride in the ash does not give the true amount of sodium chloride, on account of volatilisation during incineration.

Chlorides are estimated most readily by soaking 1-2 gm. material in dilute nitric acid for 12-18 hours in a 100 or 200 c.c. measuring flask. The chlorides diffuse out of the tissue in this time.   The volume is made up to 100 or 200 c.c., the mixture shaken and filtered into a dry vessel. The chlorides are estimated in a portion of the filtrate by Volhard's method.

(3) *Estimation of Protein.*

Protein is estimated from a nitrogen determination in 1-2 gm. (or more in case of certain vegetable foods) of material by Kjeldahl's method (p. 26).

The amount of nitrogen multiplied by the factor 6·25 gives the amount of protein.   In the case of milk, the factor 6·38 is used.   The factor 5·68 is more correct in the case of vegetable proteins.

(4) *Estimation of Fat.*

This estimation is carried out by Soxhlet's method (p. 238).   From 1-10 gm. of material are dried in a basin, transferred to a paper thimble, and extracted with ether.

In the case of milk, 5 c.c. is absorbed on a roll of fat-free filter paper contained in a weighing bottle, dried, and extracted with ether.

(5) *Estimation of Carbohydrate.*

In animal tissues, the amount of carbohydrate is negligible except in the case of heart, liver, etc. The difference between 100 and the sum of the other analysis represents carbohydrate.

In vegetable tissues, it is often necessary to distinguish cellulose from digestible carbohydrates.

Cellulose, or fibre, estimations are made by boiling 5-10 gm. (most conveniently the residue from the fat estimation) under a reflux, first with 2 per cent. hydrochloric acid, filtering off and washing, and then with 2 per cent. caustic soda, in each case for 1 hour. The insoluble residue is filtered off, washed, dried, and weighed.

The difference from the total now represents carbohydrate.

Glucose, cane sugar, etc., in fruits can be estimated by soaking a weighed portion of material in water containing 1 per cent. toluene, making up to a known volume, filtering, and estimating the reducing sugar, before and after hydrolysis, by Fehling's method.

Lactose is very conveniently and rapidly estimated in milk by taking 15 c.c. milk in a 100 c.c. flask, diluting to about 50 c.c. with water, precipitating protein and fat with 7-8 c.c. of dialysed iron, making up to volume, mixing, and filtering. The lactose in the clear filtrate is estimated by Fehling's, or other, method.

CHAPTER LII.

RESPIRATORY EXCHANGE.

THE respiratory exchange over short intervals of time, and under different conditions, can be accurately determined by the "bag method" of Douglas.

The method consists in collecting the expired air during a short time, ascertaining its composition by analysis and contrasting it with the composition of the inspired air. From these data the consumption of oxygen and the production of carbon dioxide is calculated, and hence the respiratory quotient.

The apparatus is shown in Fig. 59. It consists of (1) a wedge-
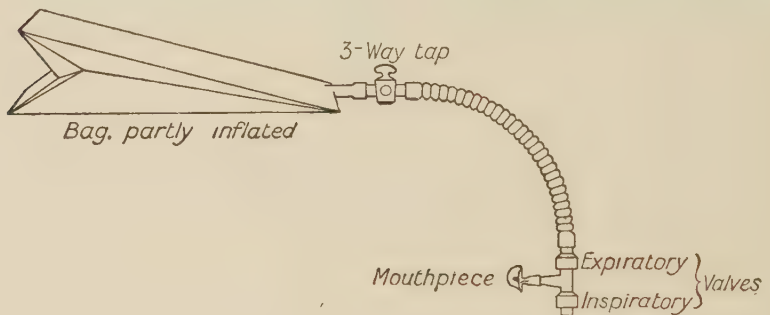
FIG. 59.

shaped gas bag made of fabric lined with rubber, capable of holding 60, or more, litres of air, (2) a large bore 3-way tap, (3) flexible tubing of wide bore, (4) mouthpiece and valves. In fitting together the various parts, it is important to see that the 3-way tap is in the correct position for turning by the subject who has the bag behind him. It is also highly important that residual air in the bag is of similar composition to expired air. This is attained by partially filling the bag, emptied by pressure, with expired air by breathing into it for 1 to 2 minutes through the valves. The bag is then completely emptied by pressing and rolling it up towards the tap. The bag is then shut by turning the tap in the

position, so that any breathing through the apparatus passes out into the air.

Collection of Expired Air.

Expired air may be collected under various conditions, e.g. at rest, walking, running, working a bicycle ergometer, after a diet of sugar. For most of these conditions the bag is carried on the back and the apparatus takes the form shown in Fig. 60.
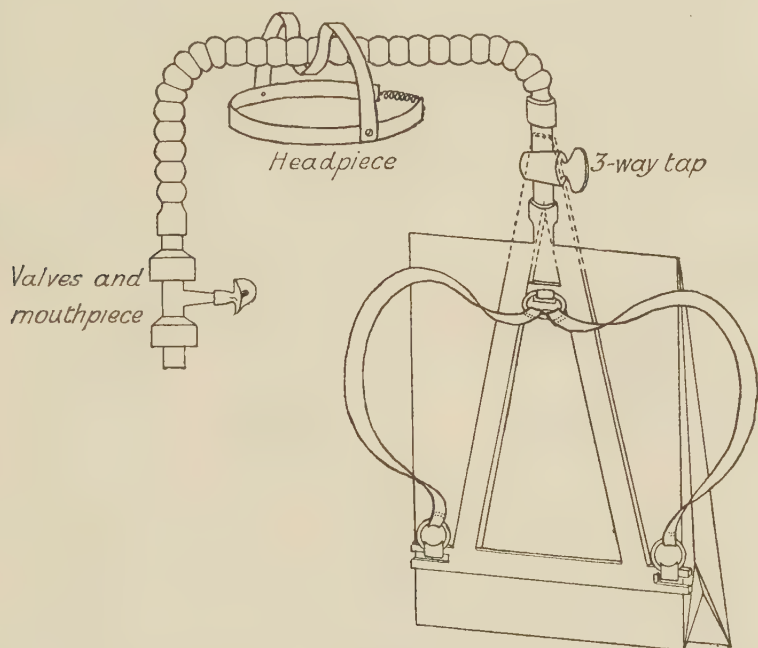
FIG. 60.
By kind permission from Douglas and Priestley's " Human Physiology."   Clarendon Press, Oxford, 1924.

(1) *At rest.*

The subject lies down, or seats himself comfortably, with the bag on a table behind him.   Ten minutes must be spent in this position to allow the metabolism to become steady.   During the second five minutes, the breathing is carried out through the valves so as to become accustomed to the apparatus.   As soon as the breathing is regular, and best at the end of an expiration, which can be felt by putting a hand over the open end of the tap, the tap is turned so that the expired air goes into the bag and the time is noted.   After 5 to 6 minutes, at the end of an expiration, the tap is again turned to shut off the bag and the time carefully noted.

(2) *At rest in post-absorptive state.*

No food is taken for 12 hours previously.   The expired air is collected as described above.

(3) *At rest after consumption of sugar.*

The expired air is taken as in (2), 75 grams of sugar, dissolved in water or tea, are quickly taken, and the expired air taken at intervals of 15 minutes.

(4) *Walking.*

The apparatus in Fig. 60 is required with a larger bag.   A steady walk must be previously practised.

Measurement of the Volume of Expired Air and Procuring of Sample for Analysis.

The mouthpiece and valves of the apparatus are disconnected and the free end attached to a wide T-piece connecting to a gas-sampling tube and gas meter as in Fig. 61.
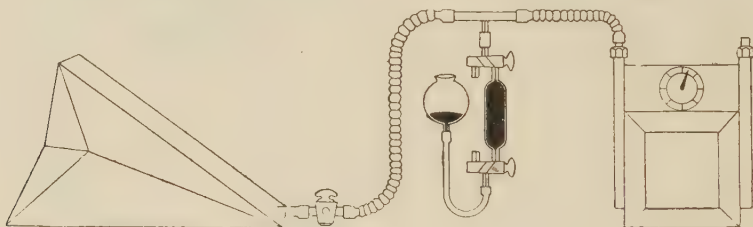
Fig. 61.

By kind permission from Douglas and Priestley's " Human Physiology."   Clarendon Press, Oxford, 1924.

Before attachment, the gas-sampling tube is filled completely with mercury, by raising the bulb with both taps open, up to the entry into the wide part of the T-tube, and one tap is closed.   The air in the bag is thoroughly mixed by pressing it with the hands.   The 3-way tap is opened and the expired air passed into the meter by gentle pressure.   After 10 litres have passed, the tap to the gas-sampling tube is opened and with steady pressure on the bag the mercury falls.   The tap is closed just before the last of the mercury leaves.   The bag is ultimately rolled up towards the 3-way tap to expel the last portions of expired air.

The readings of the gas meter indicate the volume passed in the time of collection (5·5 minutes).   This volume is reduced to the volume per minute at N.T.P., e.g. vol. = 41·2 li., T. = 13° C, Bar. = 762 mm. Tension of aqueous vapour at 13° = 11·2 mm.

$$\text{Corr. vol.} = \frac{41 \cdot 2 \times (762 - 11 \cdot 2)}{760} \times \frac{273}{286} = 38 \cdot 39 \text{ li.}$$

Vol. per. min. = 6·98 li.

Analysis of Air.

Samples of air are conveniently and rapidly analysed in the apparatus shown in Fig. 62. This is a simplified form of the standard apparatus devised by Haldane, first proposed by Guthrie and then improved by Edkins.

The apparatus consists of a gas burette with bulb of about 14 c.c. capacity holding a little more than 20 c.c. It is contained in a large glass tube (which may be filled with water to keep temperature constant). The gas burette is attached to rubber tubing and a small cylindrical bulb to act as levelling tube. At its upper end the burette is fused to a 2-way tap, which connects to air, or to the absorbing vessels through a T-tap. The absorbing vessels S and P are small inverted cylindrical cups placed in small beakers—in one of these vessels, P, there are pieces of glass tubing to facilitate absorption. The whole apparatus is fixed to a wooden stand which is painted white.

The apparatus may be adjusted as follows: The 2-way tap is turned to open to the air, and 50 per cent. glycerol, as suggested by Edkins, conveniently coloured with eosin, is put into the levelling tube. On raising the levelling tube, air is slowly driven out of the gas burette. The tube should

FIG. 62.
Edkins' modification of Guthrie's apparatus.

be raised very slowly, as the liquid fills the bulb, to avoid liquid running out of the tap. The tap is closed.

Caustic soda,[1] or potash, is put into absorbing vessel S, and alkaline pyrogallol [2] into absorbing vessel P. In order that the liquids enter the

---

[1] 20 per cent.

[2] 10 gm. pyrogallol in 100 c.c. of saturated KOH solution (160 gm. KOH in 130 c.c. water).

inverted cups, the tap T is taken out, and then returned.   Absorption vessel S is connected to the gas burette, and the levelling tube raised or lowered slowly so as to bring the potash to the mark.   It is adjusted at the mark and the T-tap is closed to this absorbing vessel.   Air received in the gas burette is driven out by raising the levelling tube, with the 2-way tap open to air.   The tap is closed and opened to the absorbing vessels.   The T-tap is opened to the gas burette and absorbing vessel P. On raising or lowering the levelling tube, liquid comes to the mark at which it is adjusted and the T-tap is shut.   The air in the gas burette is again driven out as before.   In all subsequent operations the liquids in the absorbing vessels are brought to the marks so that the volume of gas (nitrogen) in the limbs of the apparatus is kept constant.

Atmospheric air for analysis is introduced by opening the 2-way tap to the air and lowering the levelling tube.   A sample of 20 c.c. is taken ; it is measured by putting the liquid in the levelling tube at the same level as the liquid in the gas burette at the 20 c.c. mark and then closing the tap.   The temperature and pressure are noted.   The gas is first driven into the potash-absorbing vessel by opening the taps and raising the levelling tube until the water just reaches the 2-way tap. After giving time for absorption, the gas is taken back to the gas burette by lowering the levelling tube and letting the potash come to the mark. The volume is read with the level of liquid in levelling tube at the same level as in the gas burette.   The volume is noted.   The gas is driven again to the potash bulb and drawn back and volume noted.   If the volume is constant, the measurement, temperature and barometer are noted.   If not constant, the gas is drawn again to the potash and withdrawn.   The procedure is continued till the volume is constant.   The difference in volume between this and the original reading is that of the carbon dioxide.   The same procedure is carried out to drive the gas into the pyrogallol vessel.   The difference in volume gives the amount of oxygen.

These volumes are calculated to N.T.P. with the correction for tension of aqueous vapour (for values see p. 540).   Thus

$$V = \frac{v \times (\text{Bar} - T)}{760} \times \frac{273}{273 + T}$$

Pure fresh air has the composition :—

$$CO_2 = 0.03 \text{ per cent.}$$
$$O_2 = 20.93 \quad ,, \qquad ,,$$
$$N_2 = 79.04 \quad ,, \qquad ,,$$

In analysing a sample of expired air, the gas is introduced from a sampling tube. The water in the burette must be passed to the open end of the burette, and also through the connection to air of the sampling tube, so as to fill all connections completely. On turning the taps into the correct positions, the gas is introduced to the gas burette by lowering the levelling tube and its volume measured at N.T.P. It is analysed as described above and data calculated. A sample of expired air may be found to have the composition :—

$$CO_2 = 3 \cdot 60 \text{ per cent.}$$
$$O_2 = 16 \cdot 90 \quad ,, \quad ,,$$
$$N_2 = 79 \cdot 50 \quad ,, \quad ,,$$

Calculation of Results.

On comparing the analysis of pure air and expired air it will be seen that the nitrogen percentages differ. As nitrogen is not involved in respiration, the difference shows that the volume of dry air expired is less than the volume of dry air inspired. A correction must be applied before the oxygen percentages can be subtracted. The difference between the oxygen percentages is not $20 \cdot 93 - 16 \cdot 90$, but

$$\left(20 \cdot 93 \times \frac{79 \cdot 50}{79 \cdot 04} \right) - 16 \cdot 90, \text{ or } 21 \cdot 05 - 16 \cdot 90 = 4 \cdot 15.$$

A correction for carbon dioxide may be neglected.

The volume expired per minute was found to be $6 \cdot 98$ litres, hence the oxygen absorbed is

$$6980 \times \frac{4 \cdot 15}{100} = 289 \cdot 67 \text{ c.c.} = 290 \text{ c.c.}$$

and the carbon dioxide produced is

$$6980 \times \frac{3 \cdot 97}{100} = 277 \cdot 01 \text{ c.c.} = 277 \text{ c.c.}$$

The respiratory quotient is thus

$$\frac{277}{290} = 0 \cdot 955.$$

33 *

CHAPTER LIII.

EXAMINATION of urine, both qualitatively and quantitatively, gives information upon the metabolism of food, especially protein metabolism and the metabolism of mineral matter. Information is also given concerning the excretory activity of the kidney. The presence of abnormal constituents such as glucose, protein, bile pigments, and bile salts indicates some error in metabolism, or some fault in the working of the kidney. Urinary sediments must also be examined.

ANALYSIS OF NORMAL URINE.

Colour.—The colour is of a transparent yellow, which varies from light yellow to orange, or red-brown ; the froth which forms on shaking soon disappears. It contains no sediment, but on standing a cloudiness of mucoid appears containing epithelial cells.

Urine has a characteristic and peculiar odour.

Reaction.—The reaction is acid to litmus paper. This acidity is due to acid phosphates and not to free acid. On standing, a reddish-deposit of urates may form. This dissolves on warming.

At the full height of digestion urine may be slightly alkaline and may show a cloudiness due to the presence of calcium and magnesium phosphates. A white deposit then settles out on standing. It does not dissolve on warming, but dissolves in mineral acid.

Urine becomes alkaline on standing owing to the setting in of "ammoniacal fermentation" by the *micrococcus ureae*, which converts urea into ammonium carbonate. This change is in reality due to the action of the enzyme—urease—liberated from the micro-organisms.

Specific Gravity.—The usual specific gravity is 1015-1025 but it may vary from 1010-1040.

The specific gravity is ascertained with a hydrometer (urinometer).

It is advisable to use hydrometers graduated 1000-1020 and 1020-1040. The clean instrument should be put in the centre of the urine, free from froth, and the level read at the surface of the urine.

The last two figures of the specific gravity multiplied by 2·6 (Long's coefficient) give roughly the total amount of solids in 1000 c.c.

Volume.—Its volume varies. The average volume is 1000 to 1500 c.c. per day under normal conditions.

It is very advantageous to make the volume up to 2000 c.c., after taking the specific gravity and before carrying out quantitative analyses.

Acidity.

The acidity of urine is greatest on a meat diet, from the sulphuric and phosphoric acids produced by oxidation of the protein. A vegetable diet may produce a urine with alkaline reaction from the residue of inorganic bases left on oxidation of the salts of organic acids.

Estimation.

In order to estimate the acidity a sufficient amount of alkali of known strength is added from a burette until neutralisation is produced, as indicated by the colour reaction of phenolphthalein, which has been previously added. It must be remembered that different indicators give different results. Congo red and methyl orange are only affected by strong acids, whereas litmus and phenolphthalein are affected by weak acids, such as carbonic acid. The two former do not react with urine.

Procedure.

(*a*) 25 c.c. of urine are measured out with a pipette into a flask, or beaker, and diluted with 50-100 c.c. of water. About 6 drops of phenolphthalein solution are added and ·1 N sodium hydroxide is run in from a burette until the solution has a permanent and distinct pink colour. Owing to the presence of calcium salts in the urine, the end point is difficult to see.

(*b*) To overcome the difficulty, Folin recommends that the titration be carried out in the presence of neutral potassium oxalate :—

25 c.c. of urine are diluted with an equal volume of water, 15 gm. of finely powdered potassium oxalate and 4-5 drops of phenolphthalein are added. The solution is shaken for 1-2 minutes to dissolve most of the oxalate, and whilst the solution is still cold from the effect of the oxalate, it is titrated with ·1 N alkali until a permanent pink tint remains.

This neutral solution is used in the estimation of ammonia + amino acids (below).

As urine contains a mixture of acids, the result is not expressed in

terms of any particular acid, but in terms of ·1N acid, either per 100 c.c. of urine, or per 24 hours' quantity (diluted to 2000 c.c.), thus :—

Suppose 25·c.c. urine required 4·5 c.c. of ·1N alkali, the acidity is 18 c.c. per 100 c.c., or 360 c.c. ·1N per 24 hours' quantity.

Ammonia.

The amount of ammonia in urine is normally from 0·4-0·7 gram a day. Administration of inorganic acids, or their ammonium salts, increases the amount. An increase is found in starvation and in diabetes from the formation in the body of acids (aceto-acetic, etc.), if alkali is not given at the same time.

The estimation of ammonia in urine is easily made by the aeration method introduced by Folin in 1902. The urine is made alkaline with sodium carbonate and the ammonia evolved removed by suction and collected in standard acid.

The apparatus required for this estimation is the same as that used in estimating urea by means of urease (p. 131).

25 c.c. of urine are placed in the tall gas cylinder together with 1 gm. of anhydrous sodium carbonate and a few c.c. of toluene, or paraffin. The cylinder is connected to the receiver containing 10 or 20 c.c. of ·1N acid coloured with a few drops of alizarin red as indicator.

The cylinder is placed in a bath at 40° and a good air current is passed for 1 hour (longer if the suction is not rapid). The liquid in the receiver is titrated with ·1N alkali.

The results are expressed in gm. of NH_3, or better, in terms of nitrogen as ammonia per 24 hours' quantity of urine (2000 c.c.). Thus suppose 6·0 c.c. alkali were required :—

$$10 - 6·0 = 4·0 \text{ c.c. } ·1N \text{ } NH_3$$
$$= 4 \times ·0017 \text{ gm. } NH_3, \text{ or } 4 \times ·0014 \text{ gm. N as ammonia}$$
$$= 4 \times ·0017 \times \frac{2000}{25} \text{ gm. } NH_3, \text{ or } 4 \times ·0014 \times \frac{2000}{25} \text{ gm. N as ammonia per 24 hours.}$$

Various modifications of this method have been put forward, e.g. using baryta instead of sodium carbonate. In this case the amount of amino acids can be estimated in the remainder (below).

Ammonia + Amino Acids.

In 1908, Malfatti showed that the ammonia in urine could be rapidly estimated by titrating neutralised urine after the addition of formaldehyde. The method depends upon the fact that when a neutral solution of an ammonium salt is treated with formaldehyde, combination

occurs with the formation of hexamethylenetetramine (urotropin) with the liberation of a corresponding amount of acid which can be titrated with ·1 N NaOH.   The reaction is

$$4NH_4Cl + 6CH_2O = N_4(CH_2)_6 + 6H_2O + 4HCl$$
$$4HCl + 4NaOH = 4NaCl + 4H_2O.$$
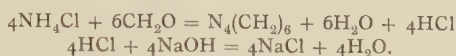
The amount so obtained is higher than the value obtained by the Folin method and is due to the presence in the urine of small amounts of amino acids which also react with formaldehyde (p. 176).   The result is therefore the amount of ammonia and amino acids.

Since the amino acids are present only in very small amounts, the value can be used as an expression of the ammonia content.   The method is particularly useful for clinical work.

Procedure.

To the neutralised urine, remaining after the acidity estimation, are added 10 c.c. of formalin which has been diluted with 2 volumes of water and neutralised with ·1 N alkali to phenolphthalein.

The pink colour of the urine disappears ; ·1 N alkali is run in from a burette until a permanent pink colour is again obtained.   The number of c.c. required is noted.

The result is expressed in gm. of NH_3, or gm. of ammonia N.

Amino Acids.

The exact estimation of the amino acids in urine is troublesome to effect.   A very close approximation is given by the difference between the ammonia and the ammonia + amino acid values.   It is more accurately obtained by performing the ammonia estimation by Folin's method, using 50 c.c. of urine and 50 c.c. of baryta water.   The remainder in the cylinder is washed into a 250 c.c. measuring flask, diluted to the mark, mixed, and filtered ; 100 c.c. portions of the filtrate are neutralised to phenolphthalein with ·2 N hydrochloric acid ; 10 c.c. of neutralised formalin are added and the solution is titrated with ·1 N alkali.

The value multiplied by $\frac{25}{20}$ is the amount in 25 c.c. of urine. Hence the amount in the 24 hours' quantity.   It is best expressed in terms of nitrogen.

Total Nitrogen.

This estimation is carried out by Kjeldahl's method as described on p. 26.

5 c.c. of urine are heated with 10 c.c. of pure sulphuric acid and a small crystal of copper sulphate till oxidation is complete.

The ammonia formed is distilled off and collected in 50 c.c. ·1N H_2SO_4.

Titration with ·1N NaOH gives the amount of ·1N NH_3 in 5 c.c. of urine.

Hence the amount of nitrogen in the 24 hours' quantity (2000 c.c.), e.g.

Suppose 100 c.c. ·1N H_2SO_4 were taken and 66 c.c. ·1N NaOH used.
Difference = 34 c.c. ·1N H_2SO_4 = 34 c.c. ·1N NH_3 = 34 c.c. ·1N nitrogen.
　　　　　 = 34 × 0·0014 gm. N in 5 c.c. of urine.
　　　　　 = 19·04 gm. in 2000 c.c.

Urea.

The reactions of urea and its isolation from urine are described on pp. 122, 126, 127.

Estimation.

(1) The most rapid method of estimating urea is the hypobromite method as described on p. 128.

5 c.c. of urine are treated with 25 c.c. of hypobromite solution and the volume of nitrogen evolved is measured.

As 354 c.c. of nitrogen at 0° and 760 mm. pressure are evolved by 1 gm. of urea or ·47 gm. of nitrogen as urea, the amount of urea, or urea nitrogen, in 5 c.c. urine can be calculated. Hence the amount in the 24 hours' volume.

Other substances such as ammonia, creatine, uric acid, evolve nitrogen under these conditions, but it is very small in comparison.

The method gives good comparative values for clinical work.

(2) The most accurate method of estimating urea is by the hydrolysis of the urea by urease.

It is carried out exactly as described on p. 131 using 5 c.c. urine and collecting the ammonia in 50 c.c. of ·1N acid containing a few drops of alizarin red.

Since the ammonia of the urine is included in this estimation its amount must be deducted.

Chlorides.

The amount of chlorides in urine depends chiefly upon the amount in the food. In starvation and in certain diseases, the chlorides may disappear from urine.

The presence of chlorides in urine is shown by acidifying with pure conc. nitric acid and adding silver nitrate. A curdy precipitate of silver chloride is formed. If the amount of chlorides is small, there is only an opalescence.

Estimation.

Chlorides are generally estimated by Volhard's method and calculated as sodium chloride. The principle of this method consists in precipitating the chlorides by excess of a standard solution of silver nitrate in the presence of nitric acid. The excess of silver is then estimated in an aliquot part of the filtrate with a solution of potassium, or ammonium, thiocyanate, which has been previously standardised against the silver solution, a ferric salt being used as indicator.

The following solutions are required :—

(1) ·1 N silver nitrate solution, or a solution of such a strength that 1 c.c. corresponds to 0·01 gm. NaCl.

(2) ·1 N potassium, or ammonium, thiocyanate solution standardised against the silver nitrate solution.

(3) Pure nitric acid free from chlorides.

(4) A saturated solution of iron alum.

Procedure.

(1) *Standardisation of the Thiocyanate Solution.*—10 c.c. of the silver nitrate solution are placed with a pipette in a beaker ; 5 c.c. of pure nitric acid, 5 c.c. of iron alum solution, and 80 c.c. of water are added. The thiocyanate solution is run in from a burette until a permanent red tinge is obtained. The amount required for the 10 c.c. silver nitrate solution is noted.

(2) *Analysis.*—10 c.c. of urine are placed with a pipette in a 100 c.c. measuring flask. About 4 c.c. of pure nitric acid and 20 c.c. with a pipette of the standard silver nitrate solution are added. The flask is filled up to the mark with distilled water, the contents are mixed and filtered into a dry vessel through a dry paper.

50 c.c. of the filtrate are taken with a pipette, some iron alum is added as indicator, and titrated with the thiocyanate solution until a permanent red colour is obtained.

The calculation of the result is :—

$$50 \text{ c.c. filtrate} = S \text{ c.c KSCN,}$$
$$\therefore 100 \text{ c.c. } \quad \text{,,} \quad = 2S \text{ c.c. KSCN,}$$
$$\text{Now } x \text{ c.c. KSCN} = 10 \text{ c.c. AgNO}_3,$$
$$\therefore 2S \text{ c.c. } \quad \text{,,} \quad = \frac{10 \times 2S}{x}.$$

This is the excess not utilised to precipitate the chlorides.

$$\therefore \left(20 - \frac{10 \times 2S}{x} \right) = \text{amount of AgNO}_3 \text{ solution used.}$$
As ·1 N AgNO$_3$ = ·1 N NaCl, 1 c.c. solution = ·00585 gm. NaCl.

Hence NaCl in gm. in the volume passed in 24 hours is calculated. An average figure is 12-15 gm. per day.

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Sulphates.

Sulphur is present in urine in three forms :—

(1) Inorganic sulphates.

(2) Ethereal sulphates.

(3) Neutral sulphur.

The sulphates are derived mainly from the sulphur in the protein; the amount of sulphate in the food is small. The average amount of sulphate in urine is 2·5 to 3·0 gm. per day. It varies directly with that of the nitrogen. The value of $\dfrac{\text{total N}}{SO_3}$ is about 5.

The ethereal sulphates increase in amount when putrefactive changes occur in the intestine, or if poisonous substances, such as phenol, be taken. It is often of great importance to know the relative amounts of inorganic and ethereal sulphates.

The neutral sulphur consists of cystine, thiocyanates, sulphides and other substances.

The amount of neutral sulphur appears to be independent of the total quantity of sulphates and probably indicates the amount of tissue which is catabolised.

The presence of inorganic sulphates is shown by acidifying with conc. hydrochloric acid and adding barium chloride solution. An opalescence indicates the presence of sulphates.

The ethereal sulphates form soluble barium salts and are not precipitated directly by barium chloride. (See p. 67.)

The presence of ethereal sulphate can only be shown after removal of the inorganic sulphates. An equal volume of baryta mixture (2 parts barium hydrate solution and 1 part barium chloride solution) is added to 10 or 20 c.c. of urine. The precipitate of barium phosphate and sulphate is filtered off. The *clear* filtrate is boiled with about $\frac{1}{4}$ its volume of conc. HCl. The ethereal sulphates are hydrolysed and a precipitate of barium sulphate is formed.

The presence of neutral sulphur is shown by oxidation after removal of inorganic and ethereal sulphates, as described under estimation.

Estimation.

The estimation of the various forms of sulphur is effected by determining (1) the amount of inorganic sulphate, (2) the amount of ethereal sulphate, after removal of the inorganic sulphate, by hydrolysis to inorganic sulphate, or by hydrolysis of the ethereal sulphate and determination of the inorganic and ethereal sulphate together and taking the difference, (3) the estimation of the total sulphates after

oxidising the neutral sulphur to sulphate.   The difference between this amount and that of inorganic + ethereal is the amount of neutral sulphur.

*   *Volumetric Estimation.*

The inorganic and ethereal sulphates may be estimated by the volumetric method proposed by Rosenheim and Drummond in 1913. It depends upon the insolubility of benzidine sulphate in very dilute hydrochloric acid.   Excess of acid hinders the precipitation.

*   (1) *Inorganic Sulphates.*

25 c.c. of urine are pipetted into a clean 250 c.c. flask, or beaker, and acidified to congo red with 1-2 c.c. of dilute hydrochloric acid ; 100 c.c. of benzidine chloride solution[1] are now added.   After 10-15 minutes, the benzidine sulphate is filtered off through a small filter and washed free from acid with water saturated with benzidine sulphate.   The precipitate, together with the paper, is returned to the flask, or beaker, about 50 c.c. of water are added and the contents of the flask are heated to about 80°.   A few drops of phenolphthalein are put in and the solution is titrated with ·1N sodium hydrate until it is red in colour.

The result is in c.c. of ·1N H_2SO_4 (1 c.c. = 0·0049 gm. H_2SO_4).

*   (2) *Inorganic + Ethereal Sulphates.*

25 c.c. of urine as above are boiled gently for 15-20 minutes with 20 c.c. of dilute hydrochloric acid to hydrolyse the ethereal sulphates.   The solution is cooled, neutralised and again acidified to congo red ; 100 c.c. of benzidine chloride solution are added.   The remainder of the process is as described under inorganic sulphates.

*   (3) *Ethereal Sulphates.*

The amount of ethereal sulphates is the difference between the amounts found in (2) and (1).

(4) *Total Sulphates.*

10 c.c. of urine are evaporated to dryness in a small basin, 5-7 cm. in diameter, on a water-bath with 5 c.c. of Denis-Benedict's oxidising re-agent (p. 524).   The basin is covered with a clock glass and the dry residue is carefully heated over a flame until the copper nitrate is completely decom-posed and converted into copper oxide.   The basin is allowed to cool and the contents are dissolved in 10 c.c. of dilute hydrochloric acid and 10 c.c. of water.   The solution is neutralised, made very faintly alkaline with caustic soda and warmed.   The precipitate of copper oxide is filtered off and

[1] This is prepared by rubbing 4 gm. of benzidine into a fine paste with about 10 c.c. of water and transferring it with about 500 c.c. of water into a 2-litre flask.   5 c.c. of concen-trated hydrochloric acid are added and the volume made up to 2000 c.c. with water.   (100 c.c. of this solution will precipitate ·1 gm. H_2SO_4.)

thoroughly washed.   To the filtrate, now of a volume of about 25 c.c., are added 1-2 c.c. of dilute hydrochloric acid and 100 c.c. of benzidine chloride solution.   The benzidine sulphate is filtered off and treated as under (1).

(5) *Neutral Sulphur.*

The difference between the amounts found in (4) and (2) is the amount of neutral sulphur.

Gravimetric Estimation.

The usual and most accurate method of determining the various forms of sulphur in urine is gravimetric.   The following procedure of Folin is the one usually adopted :—

(1) *Ethereal + Inorganic Sulphates.*

5 c.c. of 4 per cent. potassium chlorate solution and 5 c.c. of concentrated hydrochloric acid are added to 50 c.c. of urine in a 200 c.c. conical flask.   The mixture is boiled for 5-10 minutes, so as to hydrolyse the ethereal sulphates and to oxidise the pigments of the urine.   The solution becomes colourless.   25 c.c. of 10 per cent. barium chloride solution is slowly dropped in through a funnel with a capillary point.   The solution is kept just below the boiling-point for $\frac{1}{2}$-1 hour.   The barium sulphate is filtered off on filter paper, washed for half an hour with hot water, at intervals of a few minutes hot 5 per cent. ammonium chloride being substituted for the water, so that in all five or six additions of ammonium chloride take place in the course of the first 20 minutes' washings.   The filter and precipitate are dried by folding and pressing gently between dry filter papers and are transferred to a weighed crucible ; 3 or 4 c.c. of alcohol are poured into the crucible and ignited.   This dries and partially burns the filter paper.   The residue is heated to whiteness, cooled and weighed.

The barium sulphate may also be collected on a Gooch crucible.

(2) *Ethereal Sulphates.*

200 c.c. of urine are placed in a beaker and 100 c.c. of 10 per cent. barium chloride solution are added.   The mixture is stirred and set aside for 24 hours.   The clear supernatant liquid is decanted into a dry beaker and filtered. 150 c.c. of the filtrate (= 100 c.c. of urine) are measured into a 400 c.c. conical flask, 10-15 c.c. of concentrated hydrochloric acid and 10-15 c.c. of 4 per cent. potassium chlorate solution are added.   The mixture is heated to boiling.   The remainder of the operation is the same as above (1).

(3) *Total Sulphates.*

(Denis' modification of Benedict's process.)

25 c.c. of urine are placed in a porcelain basin, 4·5 cm. in diameter, and evaporated to dryness with 5 c.c. of a copper nitrate oxidising solution.[1] The residue is first gently heated with a small flame and finally to redness for 10-15 minutes.   The black residue is dissolved in 10-15 c.c. of 10 per cent. hydrochloric acid, which is gently warmed.   The solution is transferred to a 200 c.c. conical flask, diluted to 100 or 150 c.c., and precipitated with 25 c.c. of 10 per cent. barium chloride solution as above.

A blank must be made with the oxidising solution.

[1] 25 gm. copper nitrate + 25 gm. sodium chloride + 10 gm. ammonium nitrate per 100 c.c.

Phosphates.

The amount of phosphates in urine bears a direct relation to the amount of food ingested. Phosphorus compounds—phosphates, nucleic acid, lecithine, phosphoprotein—are present in all foods. The animal converts the organic compounds into inorganic phosphate. Only a small quantity of phosphorus is excreted in the urine in organic combination. The average daily excretion of P_2O_5 is about 2·5 gm.

Phosphates are present in urine mainly as acid phosphates, which give urine its acid reaction.

A precipitate of the alkaline earth phosphate may be formed on boiling urine from change of reaction due to evolution of carbon dioxide.

The presence of calcium phosphate is shown by adding ammonia to 20 c.c. urine and boiling. Calcium and magnesium phosphates are precipitated. The precipitate is filtered off, washed with water and dissolved in 5 c.c. of dilute acetic acid. One part of this solution is tested for phosphates by adding concentrated nitric acid and ammonium molybdate and boiling—a yellow precipitate of ammonium phosphomolybdate is formed. The other part is tested for calcium by adding ammonium oxalate solution—calcium oxalate is precipitated.

The presence of acid phosphates is shown by adding barium chloride solution to about 5 c.c. urine and filtering off barium phosphate. The *clear* filtrate is treated with baryta mixture and boiled :—

$$2Na_2HPO_4 + 3BaCl_2 = Ba_3(PO_4)_2 + 2HCl + 4NaCl$$
$$2NaH_2PO_4 + 3Ba(OH)_2 = Ba_3(PO_4)_2 + 2NaOH + 4H_2O.$$
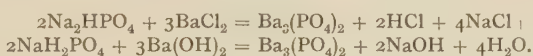
The precipitate is dissolved in nitric acid and tested for phosphates with ammonium molybdate.

Volumetric Estimation of Inorganic Phosphates.

The usual method of estimating phosphates in urine depends upon their precipitation as uranium phosphate $(UrO_2)HPO_4$ by a standard solution of uranium acetate, or uranium nitrate in the presence of sodium acetate and acetic acid :—

$$UrO_2(C_2H_3O_2)_2 + KH_2PO_4 = UrO_2HPO_4 + KC_2H_3O_2 + C_2H_4O_2.$$

The determination of the end point, at which excess of soluble uranium salt is first in solution, is shown by means of potassium ferrocyanide, or by cochineal tincture, which becomes green at this point.

The following reagents are required :—

(1) Acid sodium acetate solution. (2) Cochineal tincture.

(3) ·1N uranium solution of which 1 c.c. = 0·00355 gm. P_2O_5, or a solution of such a strength that 1 c.c. = 0·005 gm., or 5 mgm., P_2O_5.

The uranium solution cannot be prepared directly but requires to be standardised against a standard phosphate solution. Acid potassium phosphate, KH_2PO_4, is weighed out and dissolved in water, so that 50 c.c. contain 0·1 gm. P_2O_5. 50 c.c. of this solution are titrated with the uranium solution (36 gm. in 1 litre) in the manner described below ; the uranium solution is then diluted so that 1 c.c. = 5 mgm., or 3·55 mgm., of P_2O_5.

Procedure.

50 c.c. of urine are placed with a pipette in a 100 c.c. beaker, 5 c.c. of acid sodium acetate solution and a few drops of cochineal tincture are added. The urine is heated to boiling and the standard uranium acetate solution is run in slowly from a burette as long as a precipitate is formed. The solution is kept boiling and uranium solution is added, drop by drop, until the red colour is changed to green. The end point is best tested by taking out a drop of the solution and placing it in contact with a drop of potassium ferrocyanide solution, or a little heap of finely powdered ferrocyanide, on a white piece of porcelain. A brown colour, or precipitate, is formed when excess of soluble uranium salt is present in the solution. (A few more drops are generally required to reach this point than to turn the cochineal green.) The calculation of the result is :—

50 c.c. of urine = n c.c. of uranium solution = $n \times$ 0·005 gm., or $n \times$ ·00355 gm., P_2O_5. Hence the quantity of P_2O_5 in the 24 hours, quantity of urine is calculated.

Uric Acid.

The chemistry of uric acid is given on pp. 315-320.

Estimation.

In 1892 it was shown by Hopkins that uric acid could be completely precipitated from urine as ammonium urate by saturating the urine with ammonium chloride. Its amount was estimated by converting this into uric acid by the action of hydrochloric acid, dissolving the latter in sodium carbonate and titrating with standard permanganate solution. Very accurate results are obtained by Folin and Schaffer's method, which is in reality a shortened Hopkins' method. It is the one most commonly employed.

For this method the following reagents are required :—

(1) Uranium acetate mixture (see p. 546).

(2) 10 per cent. ammonium sulphate solution.

(3) ·05N potassium permanganate solution made by dissolving 1·581 gm. pure potassium permanganate in 1 litre of water (1 c.c. = 0·00375 gm. uric acid).

*   *Procedure.*

200 c.c. of urine are measured out with a pipette into a 500 c.c. flask and 50 c.c. of the uranium acetate mixture are added. The solutions are mixed and allowed to stand for about half an hour, so as to allow the precipitate to settle. This precipitate contains a mucoid substance which, if not thus removed, renders the subsequent filtration and washing of the ammonium urate precipitate very slow. The supernatant liquid is filtered off through a dry filter into a dry vessel; 125 c.c. (= 100 c.c. urine) of this are measured out with pipettes into a beaker; 5 c.c. of concentrated ammonia are added and mixed, and it is allowed to stand covered with paper for 12-24 hours.

The supernatant liquid is carefully decanted upon a filter, the precipitate of ammonium urate is washed on to the filter with 10 per cent. ammonium sulphate solution and washed once or twice with the same reagent to remove the chlorides as completely as possible.

The filter is removed from the funnel, opened, and with a fine stream of water the ammonium urate precipitate is washed into a beaker, or flask. To the ammonium urate precipitate, suspended in about 100 c.c. of water, 15 c.c. of concentrated sulphuric acid are added and it is titrated at once without cooling with ·05 N potassium permanganate until a pink colour is first seen throughout the solution (cf. p. 320).

The result is calculated as follows :—

$$1 \text{ c.c. } \cdot 05 \text{ N KMnO}_4 = 0 \cdot 00375 \text{ gm. uric acid,}$$
$$\therefore x \text{ c.c.} \qquad ,, \qquad = x \times 0 \cdot 00375 \text{ gm. uric acid,}$$

but since ammonium urate is slightly soluble a correction of 3 mgm. for every 100 c.c. of urine used must be added. The result is :—

100 c.c. urine contain $x \times \cdot 00375 + \cdot 003$ gm. uric acid, from which the amount in the 24 hours' quantity is calculated.

*   ## Creatinine.

Creatinine is a constant constituent of urine. Its amount varies little, unless it is present in large amounts in the food. Its reactions are given on p. 304.

Estimation.

Creatinine is estimated by Folin's adaptation of the Jaffé colour reaction of creatinine with picric acid and caustic soda. Folin found that a layer 8 mm. deep of ·5 N potassium bichromate solution had the same colour as a layer 8·1 mm. deep of a solution prepared from 10 mgm. of pure creatinine, picric acid and caustic soda. By comparing the colour

of an unknown solution with that of bichromate in a Duboscq colorimeter, the amount of creatinine can be determined as the colours are directly proportional.

Procedure.

10 c.c. of urine are measured with a pipette into a 500 c.c. measuring flask, 15 c.c. of saturated picric acid solution and exactly 5 c.c. of 10 per cent. sodium hydroxide are added. The mixture is allowed to stand for 5-7 minutes and diluted to 500 c.c. with water. The colour of this solution is compared with that of ·5N bichromate in a colorimeter.

The bichromate solution is placed in one of the cups of the colorimeter and the depth through which the colour is viewed is adjusted to 8 mm. by means of the screw and the vernier on the scale. The unknown is placed in the other cup. The comparison is made by altering the depth by means of the screw on this side of the instrument until the colours match. Readings should be taken by matching from too light and from too dark and the mean of 6-10 readings should be taken.

The result is calculated as follows :—

Suppose 9·5 mm. of the unknown match 8 mm. of the bichromate, then since 8·1 mm. of the bichromate correspond to 10 mgm. of creatinine and since the readings are proportional,

$$10 \times \frac{8\cdot1}{9\cdot5}, \text{ or } 8\cdot4 \text{ mgm., creatinine are present in } 10 \text{ c.c. urine.}$$

Hence the amount in the 24 hours' quantity is calculated.

Creatine + Creatinine.

Though creatine is not normally present in urine, it appears in certain pathological conditions.

It is estimated by conversion into creatinine.

The difference between the estimations of creatinine and creatine + creatinine gives the amount of creatine.

Benedict[1] gives the following procedure :—

A volume of urine containing between 7 and 12 mgm. of total creatinine is put into a small flask, or beaker, together with 10-20 c.c. of N hydrochloric acid and a pinch or two of powdered or granulated lead. The effect of the lead is to prevent pigment formation. The mixture is boiled nearly to dryness over a flame and then evaporated to dryness on a water-bath, or by holding the vessel in the hand and heating carefully. The residue is dissolved in 10 c.c. of hot water and the

[1] J. Biol. Chem., 1914, **18**, 192.

solution rinsed through a plug of cotton, or glass, wool into a 500 c.c. measuring flask ; 20-25 c.c. of picric acid solution are added and 7-8 c.c. of 10 per cent. sodium hydrate containing 5 per cent. of Rochelle salt, which prevents turbidity due to the presence of lead hydroxide. After 5-7 minutes the volume is made up to 500 c.c. and the colour compared with the standard.

Output of Nitrogenous Constituents.

The following table shows the average daily output in the urine of the various nitrogenous constituents on a fixed carbohydrate and fat diet, but variable protein diet (egg-white, meat, etc.,), the total quantity ingested corresponding to the 100 gm. standard :—

Diet.	Acidity ·1N.	P_2O_5. gm.	Ammonia. gm.	Urea. gm.	Creatinine. gm.	Uric Acid. gm.	Total N. gm.
Mixed	652	2·76	0·52	30·35	1·29	0·86	16·01
Meat	453	2·42	0·43	31·58	1·38	0·79	17·09
Egg-white	308	1·13	0·34	31·69	1·12	0·51	16·23
Egg-white + Na_2HPO_4 .	402	1·39	0·40	30·65	1·24	0·46	15·91
+ $CaCl_2$.	331	0·73	0·65	28·83	0·97	0·50	15·10
+ lecithine .	446	2·05	0·62	28·22	1·08	0·44	14·80
+ lemco .	384	2·25	0·47	31·79	1·69	0·76	16·56
+ herring roe .	778	4·41	0·86	29·19	1·27	1·16	16·53

It is more usual to express the output in grams of nitrogen and in the amount of nitrogen as percentage of the total nitrogen. These data are :—

Diet.	CORRESPONDING NITROGEN VALUES OF THE ABOVE.			AMOUNT OF NITROGEN IN PER CENT. OF THE TOTAL NITROGEN.				
	Urea N. gm.	Creatinine N. gm.	Uric Acid N. gm.	Ammonia.	Urea.	Creatinine	Uric Acid.	Undetermined.
Mixed	13·91	0·52	0·29	3·3	86·2	3·3	1·8	4·2
Meat	15·01	0·50	0·26	2·5	86·8	3·0	·1·5	4·8
Egg-white . . .	14·75	0·42	0·17	2·0	91·0	2·6	1·1	2·7
Egg-white + Na_2HPO_4 .	14·30	0·46	0·16	2·5	89·1	2·9	1·0	3·9
+ $CaCl_2$	13·46	0·37	0·17	3·5	89·1	2·4	1·1	3·5
+ lecithine .	13·15	0·41	0·15	3·6	88·9	2·8	1·0	3·5
+ lemco .	14·50	0·63	0·26	2·5	90·3	3·5	1·5	1·9
+ herring roe	13·62	0·48	0·41	3·7	84·0	2·8	2·2	5·8

The undetermined nitrogen is nitrogen which is not represented as urea, ammonia, etc. Its amount varies from about 3-6 per cent. of the total nitrogen and it consists of nitrogen present in the pigments of the urine and in unknown compounds.

THE PIGMENTS OF URINE.

(1) The chief pigment of the urine is urochrome, which gives urine its yellow colour.

(2) Urobilin is present only in small quantities, generally in the form of its chromogen, urobilinogen. Under certain pathological conditions its amount may be greatly increased. Urobilin is derived from hæmatin and is very like

hydrobilirubin, which is obtained by reduction of bilirubin.   It is identical with stercobilin, the yellow pigment of the fæces.

(3) Uroerythrin gives the pink colour to urate sediments.   Normally it is present only in small amounts, but is greatly increased in certain diseases.

(4) Hæmatoporphyrin is also only present in small amounts.

I. Urochrome and Urobilin in Normal Urine.

Normal urine shows no absorption bands with the spectroscope but only a general absorption of the violet.   Neither urochrome nor urobilinogen show absorption bands.

200-400 c.c. of urine are saturated with ammonium sulphate crystals [1] and allowed to stand a short time.   The precipitate, which consists of ammonium urate associated with the chromogen of urobilin, is filtered off and extracted with hot alcohol.   The alcoholic solution shows no absorption band, or only a very faint one ; on acidifying it, the chromogen is broken up and the absorption band of urobilin can be seen at the junction of the green and blue.

To the filtrate containing the ammonium sulphate is added 2-3 times its volume of alcohol.   The ammonium sulphate is precipitated and a clear yellow layer of alcohol containing the urochrome forms above the salt solution. It shows no absorption bands on examination with the spectroscope.

II. Pathological Urine containing Excess of Urobilin (or Normal Urine).

The urates are precipitated by saturating the urine with ammonium chloride [2] and filtered off.   The filtrate is saturated with ammonium sulphate, acidified with a drop of sulphuric acid and shaken up with a mixture of 2 parts of ether and 1 part of chloroform.   The urobilin is taken up by this solvent and shows the absorption band well.   If the ether-chloroform layer be pipetted into another test tube and shaken well with water made slightly alkaline with caustic soda, the pigment passes entirely into the alkaline solution.   A solution of urobilin in alcohol shows a green fluorescence and an absorption band in the green between b and F.

III. Pathological Urine containing a Pink Urate Sediment, Uroerythrin.

The urate sediment is filtered off.   If to a portion of this precipitate caustic soda be added, the pink colour becomes green.   If the remainder of the precipitate be dissolved in hot water and the solution extracted with acetic ester, or amyl alcohol, a pink solution is obtained which shows two absorption bands in the green ; if weak, only one.

Hæmatoporphyrin in Normal Urine.

For the detection of hæmatoporphyrin in normal urine, in which it is present as alkaline hæmatoporphyrin, at least 200-400 c.c. are necessary, but in pathological urine, where its quantity is increased, a smaller quantity can be used. The best method is that of Garrod.   To every 100 c.c. of urine are added 20 c.c. of dilute caustic soda.   This precipitates the earthy phosphates which carry down the pigment.   The urine must give a precipitate like that of normal urine, otherwise calcium phosphate in acetic acid solution must be added before precipitating.   This is obtained by precipitating a little calcium chloride with

---

[1] 80 gm. per 100 c.c.   [2] 27 gm. per 100 c.c.

sodium phosphate in a test tube. Too large a precipitate should be avoided. The earthy phosphates are allowed to settle and the supernatant liquid is poured off; they are transferred to a filter paper and washed with water (till the washings are colourless if hæmatoporphyrin free from other pigments be required; too much washing must be avoided if only a little hæmatoporphyrin be present). The precipitate is extracted with alcohol, acidified with hydrochloric acid and the solution is examined for the spectrum of acid hæmatoporphyrin.

URINARY SEDIMENTS.

The sediment is separated by means of the centrifuge and examined under the microscope.

A. IN ACID URINE.

Amorphous Deposits.

(1) Urates.

This deposit is known as "brick dust" from its pink colour (uroerythrin). It may contain crystalline forms and may dissolve completely on warming the urine.

A little of the sediment is boiled with water. It is soluble; the hot solution is acidified with hydrochloric acid and cooled. Uric acid crystallises out; the crystals are examined with the microscope and tested with the murexide reaction.

Urates may deposit from concentrated urine on cooling, as is commonly found in fevers.

The chief constituent is acid sodium urate.

(2) Calcium Oxalate.

Calcium oxalate has the appearance of dumb-bell or spheroidal bodies (envelope crystals) under the microscope. It is insoluble in strong acetic acid and ammonia, but soluble in hydrochloric acid.

(3) Bilirubin, or Hæmatoidin.

Yellow granular amorphous masses, which give Gmelin's reaction, generally consist of bilirubin.

Crystalline Deposits.

(4) Uric Acid.

This is known as cayenne pepper deposit, as it is of sandy red colour. It has a distinctive crystalline form under the microscope and gives the murexide reaction (Fig. 63).

(5) Calcium Oxalate.

This sediment consists of colourless, transparent, highly refractive octahedral crystals (envelope shaped). It is insoluble in acetic acid, but soluble in hydrochloric acid (Fig. 64).

(6) Ammonium Magnesium Phosphate.

Ammonium magnesium phosphate crystals separate in the form of knife rests from faintly acid urine (Fig. 65). They are soluble in acetic acid. The smaller crystals may resemble calcium oxalate in appearance, but are distinguished by their solubility in acetic acid.

FIG. 63.—Deposit of uric acid.
(After Funke.)
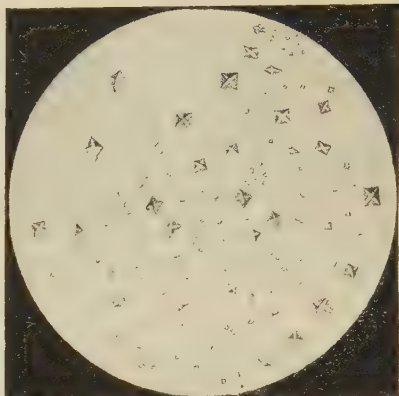
FIG. 64.—Deposit of calcium oxalate.
(After Funke.)
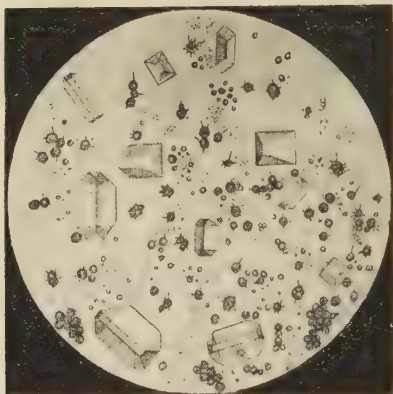
FIG. 65.—Deposit of triple phosphate
(am. mag. phosphate) and ammonium
urate. (After Funke.)

FIG. 66.—Deposit of uric acid, sodium
urate and calcium oxalate. (After
Funke.)
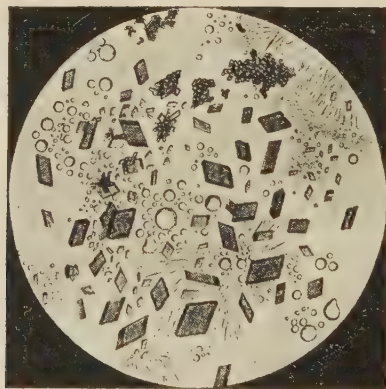
FIG. 67.—Hæmatoidin.

(7) **Calcium Hydrogen Phosphate,** $CaHPO_4 . 2H_2O.$

Large prismatic crystals, often arranged in rosettes, consist of calcium hydrogen phosphate. On adding a solution of ammonium carbonate, the crystals are eaten into and break down into an amorphous deposit. They are easily soluble in dilute acetic acid.

(8) **Bilirubin, or Hæmatoidin.**

These occur as small yellow rhombic plates which give Gmelin's reaction (Fig. 67).

(9) **Tyrosine.**

Tyrosine may be present as fine needles in star-shaped bundles (p. 211). They are insoluble in acetic acid, but soluble in ammonia and hydrochloric acid and give Millon's reaction.

(10) **Cystine.**

Cystine deposits are very rare and consist of colourless regular hexagonal plates (p. 174) which are soluble in ammonia, but insoluble in acetic acid.

B. **IN ALKALINE URINE.**

Amorphous.

(1) **Earthy Phosphates.**

Earthy phosphates appear as fine granules and are easily soluble in dilute acetic acid.

(2) **Calcium Carbonate.**

Deposits of calcium carbonate consist of fine granules or dumb-bell shaped spheroidal masses sometimes having a concentric striation. They are easily soluble in acetic acid with evolution of carbon dioxide and effervescence.

(3) **Acid Ammonium Urate.**

Spherules with small crystals adhering, "hedgehog spines," are composed of acid ammonium urate (Fig. 65). They are generally pigmented and dissolve in hydrochloric acid from which uric acid separates.

Crystalline.

(4) **Ammonium Magnesium Phosphate.**

Ammonium magnesium phosphate crystals are easily obtained from urine undergoing ammoniacal fermentation. They consist of large colourless prisms of knife-rest, or coffin-lid, shape.

(5) **Crystalline Calcium Phosphate,** $CaHPO_4.$

This deposit occurs rarely and appears as rosettes of prisms or spherules.

URINARY CALCULI.

The various constituents of urine which form sediments may take part in the formation of urinary calculi, which are of frequent occurrence and vary in size from that of a pea to that of a goose egg. They are generally coloured, commonly yellow-grey, yellow-brown, or reddish-brown. Their surface may be smooth and polished, or rough and uneven. When fractured, they appear to consist of regular concentric layers which can often be scaled off.

(1) Urate Stones.

They consist generally of ammonium urate and are of a brown colour and very hard; they are pale yellow when powdered. They give the murexide reaction and ammonia is evolved on boiling with caustic soda.

(2) Calcium Oxalate Stones.

After the urate stones these are the most frequent in occurrence. They are either smooth and small, "hempseed stones," or as large as a hen's egg with a rough and uneven surface, "mulberry stones." They cause hæmorrhage and have therefore a superficial dark brown colour. They are soluble in hydrochloric acid without effervescence, but not in acetic acid. On heating, they are converted into calcium carbonate, which dissolves in acetic acid with effervescence.

(3) Phosphate Stones.

These stones can reach a large size. They consist of calcium phosphate and triple phosphate and generally contain ammonium urate and calcium oxalate. Their colour varies; it may be white, grey, pale yellow, or even lilac (from indigo red). Their surface is uneven. They do not burn on heating; when crushed the powder is soluble in acids without effervescence and the solution gives the reactions for phosphoric acid and calcium. Ammonia is evolved from stones containing triple phosphate on heating with caustic soda.

(4) Calcium Carbonate Stones.

These stones occur chiefly in herbivora and very rarely in man. They have a chalky appearance, are white in colour and dissolve in acids with evolution of carbon dioxide.

(5) Cystine Stones.

These stones are rare and of various sizes. They may be as large as a hen's egg. Their surface is smooth or uneven, white or dull yellow, and they show a crystalline fracture; they are not very hard. On heating, they burn away almost entirely with a blue flame. They give the reaction for cystine (p. 175).

(6) Xanthine Stones.

These stones are still rarer and vary in size from a pea to a hen's egg. In colour they are dull white, yellow-brown, or red-brown. They are not very hard and they show an amorphous fracture, becoming waxy on rubbing. They are completely combustible and give a yellow murexide reaction.

(7) Urostealiths.

These stones have only been observed a few times. They are soft and elastic when wet, brittle, amorphous, and waxy when dry. They burn with a luminous flame when heated and consist of fat and cholesterol.

INBORN ERRORS OF METABOLISM.

Under this title A. E. Garrod has grouped together four anomalies in metabolism, namely, albinism, alkaptonuria, cystinuria, pentosuria. These anomalies are of life-long persistence and in most cases are inborn. They may occur as temporary phenomena in disease.

(1) **Albinism.**

The essential phenomenon of albinism is the absence of pigments of the melanin group which play the chief part in the coloration of man and the lower animals. All varieties of melanins are absent in albinos—white hair, pink eyes, unpigmented skin.

The lipochrome pigments which colour fats, serum, etc., yellow are not absent.

(2) **Alkaptonuria.**

This is the best-known example of an inborn error. It is rendered evident by the freshly passed urine of an alkaptonuric beginning to darken on exposure to the air, due to absorption of oxygen. The darkening commences at the surface and gradually spreads through the liquid until it assumes a black colour. Alkali greatly hastens the darkening.

The urine, heated with Fehling's solution, gives a deep brown colour and there is a copious reduction. The colour of the liquid differentiates it from the reduction produced by other substances. Ammoniacal silver nitrate solution is rapidly reduced in the cold. Nylander's reagent is darkened, but there is no reduction to bismuth. A yellow precipitate is formed when such urine is treated with Millon's reagent. The most striking reaction is given with ferric chloride. If the reagent be allowed to fall drop by drop into the urine, a momentary deep blue colour appears; this can be continued until oxidation is complete.

The first case of alkaptonuria was described by Marcet in 1823; in 1858 Bodeker showed that the reducing substance was not glucose. Wolkow and Baumann in 1891 clearly established that the peculiar body in the urine was homogentisic acid (quinol acetic acid, p. 216).

It is derived from the aromatic compounds—tyrosine and phenylalanine —in the protein food and probably represents a stage in their catabolism in the body.

(3) **Cystinuria.**

Cases of cystinuria are characterised by the deposits of cystine crystals in the form of hexagonal plates in the urine. The odour of hydrogen sulphide may become apparent when the urine decomposes.

The first case was that described by Wollaston in 1810, who obtained a cystine calculus from the bladder of a child. The phenomenon is believed to be the most common of the inborn errors, but is difficult of recognition.

It is also apparently due to incomplete oxidation of protein, especially the cystine unit. Frequently the diamines, putrescine and cadaverine, more rarely leucine and tyrosine, are found with cystine in the urine.

(4) **Pentosuria.**

The first case of the excretion of a pentose in the urine was described by Salkowski and Jastrowitz in 1892. Several cases have since been recorded. Though the urine reduced Fehling's solution, it did not ferment with yeast and was optically inactive. An osazone melting at 159° was isolated; glucosazone melts at 205°. The presence of pentose can be confirmed by the phloroglucinol and orcinol tests (see p. 265).

PATHOLOGICAL URINES.

(1) DIABETIC.

Diabetic urine contains glucose; β-hydroxybutyric acid, aceto-acetic acid and acetone are often present in diabetes mellitus.

Glucose.

The tests for glucose are given under carbohydrates.

Nylander's Test.

This reagent has the advantage over Fehling's by not being reduced by creatinine, or uric acid. It is reduced by glycuronic acid. 1 c.c. reagent is added to 10 c.c. urine; on heating, the solution becomes yellow, brown, dark brown; metallic bismuth finally separates out. If the quantity of sugar be small, the urine darkens, and after standing for some time, a black deposit of metallic bismuth settles out. The test will indicate 0·05 per cent. of glucose.

The Fermentation Test.

This succeeds well if the quantity of sugar be large, but if the quantity be small as indicated by Nylander's test it may not succeed. The urine is then allowed to ferment for 24-28 hours and is tested again with Nylander's reagent. If Nylander's test is now negative, glucose was originally present; if positive, the reduction is probably due to other reducing substances, e.g. glycuronic acid, lactose.

The Phenylhydrazine Test.

This test is carried out preferably as follows : to 5 c.c. of urine are added 2 c.c. of 50 per cent. acetic acid saturated with sodium acetate and 2 drops of phenylhydrazine. The solution is evaporated down to 3 c.c., cooled rapidly, again warmed and then allowed to cool slowly. Crystals separate out even if there be a very small percentage of glucose (Neumann).

The Polarisation Test.

The polarimeter distinguishes between glucose, fructose, and conjugated glycuronic acid, which is laevorotatory. If the urine be highly coloured, it is first precipitated with lead acetate (10 c.c. of a 25 per cent. solution for every 50 c.c. of urine) and the filtrate examined. The volumes must be known, if the glucose is to be estimated by the polarimeter.

The estimation of glucose is most usually carried out by Benedict's method; the fermentation method (Lohnstein) is convenient.

Aceto-Acetic Acid.

Aceto-acetic acid is tested for as follows :—

(1) To some of the urine is added dilute ferric chloride as long as a precipitate of ferric phosphate continues to form. This is filtered off and to the filtrate a few more drops of ferric chloride are added. If aceto-acetic acid be present, the colour becomes like claret.

(2) Some urine is acidified with sulphuric acid and shaken up with ether. The ether is poured off into another test tube and shaken with ferric chloride solution. A red colour is produced, if aceto-acetic acid be present.

(3) On heating with dilute alkali or acid, the aceto-acetic acid is decomposed yielding acetone ; this may be detected by its odour, but is more certainly detected by distilling and examining for acetone the first 20 c.c. of the distillate from 250 c.c. urine by the following tests :—

(a) Iodoform test : NaOH + I in KI. The precipitate should be examined with a microscope.

(b) Alcoholic solution of I + NH_3. Iodoform and a black precipitate of nitrogen iodide, which disappears gradually leaving the iodoform, are formed.

(c) Legal's test. A few drops of freshly prepared sodium nitro-prusside solution are added and it is rendered alkaline with caustic soda. A deep-red colour is formed. If acidified with acetic acid, the colour becomes reddish-purple.

β-Hydroxybutyric Acid.

It is difficult to prove the presence of β-hydroxybutyric acid in urine. If a large amount be present, the urine will show laevorotation after fermentation of the glucose. Its presence can only be shown with certainty by extraction.

(2) PROTEIN.

A. Coagulable Protein. ("Albumin.")

Most commonly coagulable protein is found and tested for by :—

(1) *Heat Coagulation.*

This is best performed by heating after adding to a little of the filtered urine one-sixth of its volume of saturated sodium chloride. The precipitate which is formed may consist of coagulated protein, or earthy phosphates, or both. The precipitate of earthy phosphates dissolves, whilst the coagulated protein separates out in flakes, if 2 drops of 33 per cent. acetic acid be added for every 10 c.c. urine.

(2) Heller's Test.

Some urine is poured on to the surface of a little concentrated nitric acid in a test tube, taking care that the two solutions do not mix. To prevent this the nitric acid may be saturated with ammonium nitrate. If protein be present, a whitish ring will form at the junction. There may be also a reddish ring due to indigo red and indigo blue. If the urine be concentrated, urea nitrate may separate out, but here the precipitate is obviously crystalline. Uric acid may also separate if a large quantity of urates be present. This can be prevented by diluting before testing with the nitric acid.

(3) Sulphosalicylic Acid.

To a few c.c. of clear urine, a *small* quantity of solid, or a few drops of a 20 per cent. solution of, sulphosalicylic acid are added. A precipitate, or cloudiness, is formed.

Other reagents for precipitating proteins can also be used.

(4) The Colour Reactions cannot be applied directly to urine, but to the heat coagulum suspended in water. Millon's reagent and the biuret reaction should be tried ; the coagulum in the latter case is dissolved in hot caustic soda.

Characterisation and Separation of a Mixture of Proteins.

(a) Albumin and Globulin.—The globulin is precipitated by half-saturating the urine with ammonium sulphate and filtered off. It is dissolved in 2 per cent. sodium chloride solution. The solution is acidified and heated.

The albumin in the filtrate is coagulated on acidifying with acetic acid and heating.

The protein nature of these precipitates should be confirmed by the colour tests.

(b) Proteose and Coagulable Protein.—The solution is saturated with ammonium sulphate (8 parts to 10 parts urine) and heated for a few seconds, so that the coagulable protein is coagulated. The precipitate is filtered off and extracted with alcohol to remove urobilin, which also gives the biuret reaction. It is extracted with boiling water which dissolves the proteose. This solution is tested with the biuret, xanthoproteic, and Millon's reactions.

(5) Estimation.

The protein is estimated by Esbach's method (see under proteins, p. 375). The urine must be acidified with acetic acid, if it is not acid, and diluted till its specific gravity is 1·006-1·008.

B. The Bence-Jones Protein.

In rare cases of disease of the bone-marrow, a peculiar protein, the Bence-Jones protein, appears in the urine.

This protein is precipitated on heating the urine to 50-60° and redissolves, more or less completely, according to the reaction and the amount of salt in the urine, when the heating is continued to the boiling-point. The

protein is not dialysable and can be precipitated by adding double the volume of saturated ammonium sulphate solution, or alcohol. The exact nature of the body is unknown but it has resemblances to globulin and proteose and yields the same amino acids on hydrolysis.

(3) **BLOOD.**

The appearance of the urine may be reddish. The urine is centrifuged to separate corpuscles and these are examined with the miscroscope.

The guaiac reaction (p. 475) may be tried and the solution is examined with the spectroscope.

Hæmochromogen is prepared by boiling with caustic soda, cooling, and reducing with ammonium sulphide ; this pigment shows the absorption bands when the other blood pigments do not (see under hæmoglobin).

The pigment can be precipitated with the earthy phosphates by caustic soda.

(4) **BILE.**

Bile pigments are tested for as follows :—

The urine is filtered through paper and Gmelin's reaction is performed.

Huppert's reaction is convenient for small quantities.

$\left.\right\}$ See under bile.

If some tincture of iodine be poured carefully upon some urine in a test tube, a green ring appears at the junction of the liquids.

Bile acids are tested for by

(1) Pettenkofer's test (after concentration if necessary).
(2) Hay's test.
(3) Oliver's test.

$\left.\right\}$ See under bile.

TABLES.

TABLE OF PRESSURE OF AQUEOUS VAPOUR (REGNAULT)

Temperature C.	Pressure in mm. of Mercury.	Temperature C.	Pressure in mm. of Mercury.
0·0	4·6	15·0	12·7
0·5	4·8	15·5	13·1
1·0	4·9	16·0	13·5
1·5	5·1	16·5	14·0
2·0	5·3	17·0	14·4
2·5	5·5	17·5	14·9
3·0	5·7	18·0	15·4
3·5	5·9	18·5	15·8
4·0	6·1	19·0	16·3
4·5	6·3	19·5	16·9
5·0	6·5	20·0	17·4
5·5	6·8	20·5	17·9
6·0	7·0	21·0	18·5
6·5	7·2	21·5	19·1
7·0	7·5	22·0	19·7
7·5	7·8	22·5	20·3
8·0	8·0	23·0	20·9
8·5	8·3	23·5	21·5
9·0	8·6	24·0	22·2
9·5	8·9	24·5	22·9
10·0	9·2	25·0	23·5
10·5	9·5	25·5	24·3
11·0	9·8	26·0	25·0
11·5	10·1	26·5	25·7
12·0	10·5	27·0	26·5
12·5	10·8	27·5	27·3
13·0	11·2	28·0	28·1
13·5	11·5	28·5	28·9
14·0	11·9	29·0	29·8
14·5	12·3	29·5	30·7

TABLE OF METRIC AND ENGLISH WEIGHTS AND MEASURES.

1 cm.	= ·375 inch.	1 inch	= 2·55 cm.
1 metre	= 1·0904 yards.	1 foot	= ·305 metre.
	= 39·37 inches.	1 yard	= ·9144 metre.

1 gm.	= 15·4 grains.	1 grain	= 64·8 milligm.
	= ·0353 oz.		= ·065 gm.
	= ·032 oz. (Troy)	1 oz.	= 28·35 gm.
	= 15 grains (Troy).	1 oz. (Troy)	= 31·1 gm.
1 kilo.	= 2·2046 lbs.	1 lb.	= 453·6 gm.

1 c.c.	= 16·89 minims.	(1 grain) = 1 minim	= ·059 c.c.
	= ·28 drachm.	20 minims = 1 scruple	= 1·18 c.c.
	= ·035 oz.	3 scruples = 1 drachm	= 3·55 c.c.
1 litre	= 1·76 pints.	8 drachms = 1 oz.	= 28·42 c.c.
	= 35·2 oz.	20 oz. = 1 pint	= ·57 litre.
	= ·22 gallon.	160 oz. = 1 gallon	= 4·546 litres.

1 gallon	= 277·27 cubic inches (water).
	= 10 lbs.
	= 70,000 grains.
1 cubic foot (water)	= 62·42 lbs.
1 fluid oz.	= 1 oz. Avoirdupois.
1 fluid drachm	= 2 drams Avoirdupois.
1 lb.	= 7000 grains.

TABLE OF INTERNATIONAL ATOMIC WEIGHTS.

				$O = 16$					$O = 16$
Aluminium	.	.	Al	27·1	Manganese	.	.	Mn	54·93
Antimony	.	.	Sb	120·2	Mercury	.	.	Hg	200·6
Arsenic	.	.	As	74·96	Molybdenum	.	.	Mo	96·0
Barium	.	.	Ba	137·37	Nickel	.	.	Ni	58·68
Bismuth	.	.	Bi	208·0	Nitrogen	.	.	N	14·01
Boron	.	.	B	11·0	Osmium	.	.	Os	190·9
Bromine	.	.	Br	79·92	Oxygen	.	.	O	16·00
Cadmium	.	.	Cd	112·40	Palladium	.	.	Pd	106·7
Calcium	.	.	Ca	40·07	Phosphorus	.	.	P	31·04
Carbon	.	.	C	12·00	Platinum	.	.	Pt	195·2
Chlorine	.	.	Cl	35·46	Potassium	.	.	K	39·10
Chromium	.	.	Cr	52·0	Selenium	.	.	Se	79·2
Cobalt	.	.	Co	58·97	Silicon	.	.	Si	28·3
Copper	.	.	Cu	63·57	Silver	.	.	Ag	107·88
Fluorine	.	.	F	19·0	Sodium	.	.	Na	23·00
Glucinum	.	.	Gl	9·1	Strontium	.	.	Sr	87·63
Gold	.	.	Au	197·2	Sulphur	.	.	S	32·07
Hydrogen	.	.	H	1·008	Tantalum	.	.	Ta	181·5
Iodine	.	.	I	126·92	Tellurium	.	.	Te	127·5
Iridium	.	.	Ir	193·1	Tin	.	.	Sn	119·0
Iron	.	.	Fe	55·84	Titanium	.	.	Ti	48·1
Lanthanum	.	.	La	139·0	Tungsten	.	.	W	184·0
Lead	.	.	Pb	207·10	Uranium	.	.	U	238·5
Lithium	.	.	Li	6·94	Zinc	.	.	Zn	65·37
Magnesium	.	.	Mg	24·32					

LIST OF REAGENTS.

ACIDS.

Acetic acid, glacial, sp. gr. 1·06 . . . . 111·1 gm. CH_3COOH

Acetic acid, dilute, $\frac{1}{2}$ normal . . . . . 3·0 gm. CH_3COOH
    (27 c.c. glacial acetic acid made up to 1 litre)

Hydrochloric acid, concentrated, sp. gr. 1·16 . . 36·6 gm. HCl

Hydrochloric acid, dilute, $\frac{2}{1}$ normal . . . 7·3 gm. HCl
    (200 c.c. conc. acid made up to 1 litre)

Hydrochloric acid, dilute, $\frac{1}{10}$ normal . . . 0·36 gm. HCl
    (10 c.c. conc. acid made up to 1 litre)

Nitric acid, fuming. sp. gr. 1·50 (about 65 per cent.)

Nitric acid, concentrated, sp. gr. 1·414 . . . 68·0 gm. HNO_3

Nitric acid, dilute, $\frac{2}{1}$ normal . . . . . 12·6 gm. HNO_3
    (125 c.c. conc. acid made up to 1 litre)

Sulphuric acid, concentrated, sp. gr. 1·84 . . 175·9 gm. H_2SO_4

Sulphuric acid, dilute, $\frac{2}{1}$ normal . . . . 9·8 gm. H_2SO_4
    (56 c.c. conc. sulphuric acid made up to 1 litre)

Sulphuric acid, dilute, $\frac{1}{10}$ normal . . . . 0·5 gm. H_2SO_4
    (2·8 c.c. conc. sulphuric acid made up to 1 litre)

ALKALIES.

Ammonia, concentrated, sp. gr. ·880 . . . 31·0 gm. NH_3

Ammonia, dilute, $\frac{2}{1}$ normal . . . . . 3·4 gm. NH_3
    (105 c.c. conc. ammonia made up to 1 litre)

Barium hydroxide, $\frac{1}{4}$ normal . . . . . 4·2 gm. $Ba(OH)_2$
    (40 gm. $Ba(OH)_2 . 8H_2O$ dissolved in water and
      made up to 1 litre)

Sodium hydroxide, sp. gr. 1·34 . . . . 40 gm. NaOH
    (410 gm. 98 per cent. caustic soda dissolved in
      water and made up to 1 litre)
    (426 gm. 94 per cent. caustic soda dissolved in
      water and made up to 1 litre)

Sodium hydroxide, dilute, $\frac{2}{1}$ normal . . . . 8·0 gm. NaOH
    (82 gm. 98 per cent. caustic soda dissolved in
      water and made up to 1 litre)
    (85 gm. 94 per cent. caustic soda dissolved in
      water and made up to 1 litre)

100 c.c. contain

Sodium hydroxide, dilute, $\frac{1}{10}$ normal . . .   0·4 gm. NaOH
    (4·1 gm. 98 per cent. caustic soda dissolved in
      water and made up to 1 litre)
    (4·2 gm. 94 per cent. caustic soda dissolved in
      water and made up to 1 litre)

SALT SOLUTIONS.

100 c.c. contain

Ammonium chloride, $\frac{2}{1}$ normal . . . .   10·7 gm.
   (107 gm. NH$_4$Cl dissolved in water and made up to 1 litre)

Ammonium carbonate, $\frac{1}{1}$ normal . . . . . .   4·8 gm.
   (48 gm. (NH$_4$)$_2$CO$_3$ dissolved in water and made up to 1 litre)

Ammonium molybdate . . . .   11·5 gm.
   (124 gm. (NH$_4$)$_6$Mo$_7$O$_{24}$. 4H$_2$O dissolved in cold water and
     made up to 1 litre)

Ammonium nitrate, $\frac{10}{1}$ normal . . . . . .   80 gm.
   (800 gm. NH$_4$NO$_3$ dissolved in water and made up to 1 litre)

Ammonium sulphide, $\frac{2}{1}$ normal . . . . .   6·8 gm.

Barium chloride, $\frac{1}{1}$ normal . . . .   10·4 gm.
   (122 gm. BaCl$_2$. 2H$_2$O dissolved in water and made up to 1 litre)

Calcium chloride, $\frac{1}{2}$ normal . . . .   2·7 gm.
   (55 gm. CaCl$_2$. 6H$_2$O dissolved in water and made up to 1 litre)

Copper sulphate (Fehling's solution A), about $\frac{1}{2}$ normal . .   4·0 gm.
   (69·3 gm. CuSO$_4$. 5H$_2$O dissolved in water and made up to
     1 litre)

Ferric chloride, $\frac{1}{2}$ normal . . . .   2·7 gm.
   (27 gm. dissolved in water and made up to 1 litre)

Iodine solution, $\frac{1}{10}$ normal . . . .   1·3 gm.
   (13 gm. iodine dissolved in 30 gm. KI in 250 c.c. water and
     made up to 1 litre)

Lead acetate, $\frac{1}{2}$ normal . . . .   8·1 gm.
   (95 gm. Pb(C$_2$H$_3$O$_2$)$_2$. 3H$_2$O dissolved in water and made up
     to 1 litre)

Lead acetate, basic $\frac{1}{2}$ normal . . . .   13·6 gm.
   (56 gm. litharge dissolved in 95 gm. lead acetate in about
     800 c.c. water and then made up to 1 litre)

Mercuric chloride, $\frac{1}{2}$ normal . . . .   6·8 gm.
   (68 gm. HgCl$_2$ dissolved in water and made up to 1 litre)

Potassium bichromate, $\frac{1}{2}$ normal . . . . . .   2·4 gm.
   (24·55 gm. K$_2$Cr$_2$O$_7$ dissolved in water and made up to 1 litre)

Potassium chloride, $\frac{1}{1}$ normal . . . .   7·4 gm.
   (74·5 gm. KCl dissolved in water and made up to 1 litre)

Potassium ferrocyanide, $\frac{1}{2}$ normal . . . . . .   4·6 gm.
   (53 gm. K$_4$Fe(CN)$_6$. 3H$_2$O dissolved in water and made up to
     1 litre)

Potassium ferricyanide, $\frac{2}{1}$ normal . . . . . .   4·4 gm.
   (44 gm. K$_3$Fe(CN)$_6$ dissolved in water and made up to 1 litre)

100 c.c. contain

Potassium oxalate, $\frac{1}{10}$ normal . . . . 0·8 gm.
    (8·3 gm. $K_2C_2O_4$ dissolved in water and made up to 1 litre)

Potassium permanganate, $\frac{1}{20}$ normal . . . . . . 1·6 gm.
    (1·581 gm. $KMnO_4$ dissolved in water and made up to 1 litre)

Potassium thiocyanate, $\frac{1}{10}$ normal . . . . . . 1·0 gm.
    (9·73 gm. KSCN dissolved in water and made up to 1 litre)

Sodium carbonate, $\frac{1}{2}$ normal . . . . 2·6 gm.
    (71·5 gm. $Na_2CO_3 . 10H_2O$ dissolved in water and made up to
    1 litre)

Sodium chloride, $\frac{2}{1}$ normal . . . . 11·7 gm.
    (117 gm. NaCl dissolved in water and made up to 1 litre)

Silver nitrate, $\frac{1}{10}$ normal . . . . 1·7 gm.
    (17 gm. $AgNO_3$ dissolved in water and made up to 1 litre)

Sodium nitrite, $\frac{1}{10}$ normal . . . . 0·7 gm.
    (6·9 gm. $NaNO_2$ dissolved in water and made up to 1 litre)

Sodium phosphate, $\frac{1}{1}$ normal . . . . 4·7 gm.
    (119·5 gm. $Na_2HPO_4 . 12H_2O$ dissolved in water and made up
    to 1 litre)

Uranium acetate, $\frac{1}{10}$ normal . . . . 2·1 gm.
    (21·3 gm. $UrO_2(C_2H_3O_2)_2 . 2H_2O$ dissolved in water and made
    up to 1 litre) (1 c.c. = 0·00355 gm. P_2O_5)

Uranium nitrate, $\frac{1}{10}$ normal . . . . 2·5 gm.
    (25·18 gm. $UrO_2(NO_3)_2 . 6H_2O$ dissolved in water and made up
    to 1 litre) (1 c.c. = 0·00355 gm. P_2O_5)

SOLIDS.

Ammonium carbonate.	Phosphorus pentachloride.
Ammonium chloride.	Phosphorus pentoxide.
Ammonium sulphate.	Potassium bichromate.
Barium carbonate.	Potassium carbonate.
Bismuth subnitrate.	Potassium oxalate.
Borax.	Potassium sulphate.
Bleaching powder.	Potassium sulphate (acid).
Calcium carbonate.	Potassium permanganate.
Calcium chloride (dry).	Soda, caustic.
Calcium sulphate (plaster of Paris).	Soda, lime.
Copper carbonate.	Sodium acetate, crystal.
Copper oxide.	Sodium acetate, fused.
Copper turnings.	Sodium carbonate (dry).
Ferrous sulphate.	Sodium chloride.
Fusion mixture (3 parts KNO_3, 1 part Na_2CO_3).	Sodium (metallic).
Lime.	Sodium nitrite.
Litharge.	Sodium nitroprusside.
Magnesium oxide.	Sodium sulphate.
Magnesium powder.	Stannous chloride.
Magnesium sulphate.	Sulphur, flowers of.
	Zinc carbonate.

SPECIAL REAGENTS.

Acid sodium acetate. 100 gm. sodium acetate ⎰are dissolved in water and
30 c.c. glacial acetic acid ⎱ made up to 1 litre.

Alcoholic caustic soda. 20 gm. sodium, or ⎰are dissolved in alcohol and made
20 gm. caustic soda ⎱ up to 1 litre.

Ammonium sulphate solution (sat.). 780 gm. ammonium sulphate are dissolved in water and
made up to 1 litre.

Barfoed's reagent. 66 gm. cupric acetate ⎰are dissolved in water and
10 c.c. glacial acetic acid ⎱ made up to 1 litre.

Benedict's qualitative reagent for glucose, etc. 173 gm. sodium citrate
100 gm. anhydrous sodium carbonate ⎱ are dissolved in about 600 c.c. water, filtered into a 1 litre measuring cylinder and diluted to about 850 c.c.

17·3 gm. $CuSO_4 . 5H_2O$ are dissolved in 100 c.c. water and diluted to 150 c.c. This solution is added with constant stirring to the citrate-carbonate solution contained in a beaker. The mixture is immediately ready for use.

Benedict's quantitative reagent for glucose, etc. 200 gm. $Na_2CO_3 . 10H_2O$
or
100 gm. Na_2CO_3
200 gm. sodium citrate
125 gm. KSCN ⎱ are dissolved with the aid of heat in enough water to make about 800 c.c. volume, and filtered.

Exactly 18 gm. of pure $CuSO_4 . 5H_2O$ are dissolved in 100 c.c. water and poured into the above solution with constant stirring. 5 c.c. of 5 per cent. potassium ferrocyanide solution are added and the whole diluted to exactly 1 litre.

The addition of the trace of ferrocyanide prevents precipitation of red cuprous oxide. The solution apparently keeps indefinitely.

Bial's reagent for pentoses. 1 gm. orcinol is dissolved in 500 c.c. of 30 per cent. HCl to which 30 drops of 10 per cent. ferric chloride have been added.

Bromine water. 25 c.c. bromine in 1000 c.c. water.

Brücke's reagent. 50 gm. potassium iodide in 500 c.c. water are saturated with mercuric iodide (120 gm.) and made up to 1 litre.

Esbach's reagent. 10 gm. picric acid ⎰are dissolved in water and made up
20 gm. citric acid ⎱ to 1 litre.

Fehling's solution. Equal volumes of A and B.
A. 69·28 gm. copper sulphate are dissolved in water and made up to 1 litre.
B. 346 gm. Rochelle salt (NaK tartrate)
130 gm. caustic soda ⎰ are dissolved in water and made up to 1 litre.

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Folins' uranium acetate mixture (uric acid).

500 gm. ammonium sulphate   are dissolved in 650 c.c.
5 gm. uranium acetate   water. The volume is
6 c.c. glacial acetic acid   about 1 litre.

Formalin.

Commercial 40 per cent. solution of formaldehyde.

Glyoxylic acid solution.

10 gm. magnesium powder are covered with water; 250 c.c. sat. oxalic acid solution are added slowly, and the solution kept cool. The magnesium oxalate is filtered off and the solution is acidified with acetic acid and made up to 1 litre.

Guaiacum tincture.

1 gm. of guaiacum is dissolved in 100 c.c. alcohol.

Gunzberg's reagent.

4 gm. phloroglucin   are dissolved in 100 c.c. absolute
2 gm. vanillin   alcohol.

Harrison's indicator.

A pinch of starch is boiled with a few c.c of water and to it is added 100 c.c. of freshly prepared 10 per cent. KI solution. This solution does not keep for more than 2 or 3 hours.

Hydrogen peroxide.

10 vols. commercial.

Iron alum solution (sat.).

300 gm. iron alum in 1 litre of water.

Magnesia mixture.

55 gm. magnesium chloride   are dissolved in water
70 gm. ammonium chloride   and made up to 1 litre.
125 c.c. ammonia (sp. gr. ·880)

Magnesium sulphate solution (sat.).

600 gm. cryst. magnesium sulphate are dissolved in water and made up to 1 litre.

Mercuric nitrate.

10 gm. mercuric nitrate are dissolved in water and made up to 1 litre.

Millon's reagent.

400 gm. mercury (= 30 c.c.) are dissolved in 570 c.c. conc. nitric acid. The solution is then diluted with 2 volumes of water.

α-Naphthol solution.

144 gm. α-naphthol are dissolved in alcohol and made up to 1 litre with alcohol.

Nylander's solution.

40 gm. Rochelle salt   are dissolved in 1000 c.c.
20 gm. bismuth subnitrate   caustic soda (8 per cent.).

Obermayer's reagent.

4 gm. ferric chloride are dissolved in 1 litre conc. hydrochloric acid.

Oil of turpentine.

Commercial.

Oxalic acid solution (sat.).

100 gm. oxalic acid are dissolved in 1 litre of water.

Pavy's solution.

120 c.c. Fehling's solution   are made up to 1 litre
300 c.c. ammonia (sp. gr. ·880)   with water.

Phenol solution.

20 gm. carbolic acid are dissolved in 1 litre of water.

Phosphotungstic acid solution. 50 gm. phosphotungstic acid ⎫ are dissolved in water and 30 c.c. conc. sulphuric acid ⎭ made up to 1 litre.

Picric acid solution (sat.). 12 gm. picric acid are dissolved in water and made up to 1 litre.

Schweitzer's reagent. Ammonium chloride and caustic soda are added to a solution of copper sulphate. The blue precipitate is filtered off, washed, pressed, and dissolved in ammonia of sp. gr. ·92.

Sodium bisulphite solution (sat.). 600 gm. sodium bisulphite are dissolved in water and made up to 1 litre.

Sodium chloride solution (sat.). 370 gm. sodium chloride are dissolved in water and made up to 1 litre.

Sodium hypobromite solution. 25 c.c. bromine are added to 100 gm. caustic soda dissolved in 250 c.c. water.

Sodium sulphate solution : sp. gr. 1030. 40 gm. sodium sulphate are dissolved in 520 c.c. water.

sp. gr. 1035.	45 gm.	,,	,,	,,	510 c.c. ,,
sp. gr. 1040.	50 gm.	,,	,,	,,	500 c.c. ,,
sp. gr. 1045.	55 gm.	,,	,,	,,	500 c.c. ,,
sp. gr. 1050.	70 gm.	,,	,,	,,	500 c.c. ,,
sp. gr. 1055.	85 gm.	,,	,,	,,	500 c.c. ,,
sp. gr. 1060.	90 gm.	,,	,,	,,	500 c.c. ,,
sp. gr. 1065.	95 gm.	,,	,,	,,	500 c.c. ,,
sp. gr. 1070.	100 gm.	,,	,,	,,	500 c.c. ,,

Stoke's reagent. 30 gm. ferrous sulphate ⎫ are dissolved in water and 20 gm. tartaric acid ⎭ made up to 1 litre. When required for use, strong ammonia is added until the precipitate first formed is redissolved.

Sulphosalicylic Acid. 20 gm. are dissolved in water and made up to 100 c.c. It may be prepared by dissolving 13 gm. salicylic acid in 10 c.c. conc. H_2SO_4 by warming, and after cooling diluting to 100 c.c.

Tannic acid solution. 100 gm. tannic acid 25 gm. sodium acetate ⎫ are dissolved in water and 25 gm. sodium chloride ⎬ made up to 1 litre. 50 c.c. glacial acetic acid ⎭

Tartaric acid solution (sat.). 750 gm. tartaric acid are dissolved in water and made up to 1 litre.

Trichloracetic acid solution. 100 gm. trichloracetic acid are dissolved in water and made up to 1 litre.

Uffelmann's reagent. Ferric chloride solution is added to 2 per cent. phenol solution till it is of a violet colour.

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ORGANIC REAGENTS.

Acetic anhydride.

Acetone.

Alcohol.

Amyl alcohol.

Benzoyl chloride.

Chloroform.

Ether.

Glycerol.

Orcinol.

Petroleum.

Phenylhydrazine.

Phenylhydrazine hydrochloride.

Phloroglucinol.

Resorcinol.

Toluene.

INDICATORS.

Alizarin red. 10 gm. sodium alizarin sulphonate are dissolved in water and made up to 1 litre = 1 per cent.

Cochineal tincture. 5 gm. cochineal are extracted with 150 c.c. alcohol + 100 c.c. water for several days ; the solution is then filtered.

Congo red. 1 gm. congo red is dissolved in water and made up to 1 litre = 0·1 per cent.

Litmus. 10 gm. litmus are finely powdered and extracted with 50 c.c. hot water.   The blue liquid is decanted and made up to 1 litre = 1 per cent.

Methyl orange. 1 gm. methyl orange is dissolved in 500 c.c. alcohol and made up to 1 litre with water = 0·1 per cent.

Methyl violet. 1 gm. methyl violet is dissolved in water and made up to 1 litre = 0·1 per cent.

Phenolphthalein. 10 gm. phenolphthalein are dissolved in alcohol and made up to 1 litre with alcohol = 1 per cent.

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